UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF PENNSYLVANIA

STEVEN HOUSER, PH.D., FAHA 3500 N. Broad Street Philadelphia, PA 19140, *Plaintiff,* v. TEMPLE UNIVERSITY 1330 Polett Walk Philadelphia, PA 19122 AND ARTHUR M. FELDMAN, MD, PHD 3500 N. Broad Street Philadelphia, PA 19140, *Defendants.*	Civil Action No. 2:21-cv-00676-WB JURY TRIAL DEMANDED

AMENDED COMPLAINT

1. Plaintiff, Steven Houser, Ph.D., FAHA ("Dr. Houser"), complains against Defendants, Temple University ("Temple") and Arthur M. Feldman, MD, PhD ("Feldman") (collectively sometimes hereinafter "Defendants"), for various claims including breach of contract, misappropriation of trade secrets, defamation, and intentional interference with contractual relations because Defendants have engaged in a scheme to defraud and defame Dr. Houser, capitalizing on Dr. Houser's research which was crucial to the formation of a new biopharmaceutical company. Feldman and Temple stand to reap millions of dollars in profits off Dr. Houser's work. To cover up their misconduct, Temple concocted pretextual "investigations" – the likes of which are not authorized by its own policies – against Dr. Houser, taking years to conduct, refusing to identify the basis of the alleged "charges" and threatening him with discipline if he did not cooperate.

2. As a result of their wrongful conduct, Defendants have caused substantial damages to Dr. Houser, for which they are liable.

Parties

3. Dr. Houser is an individual with a principal address at the above-referenced location.

4. Temple is a state-related public research university located in Philadelphia, Pennsylvania with an address at the above-referenced location.

5. Feldman is an adult individual with an address at the above-referenced location and Professor at Temple in the Department of Medicine and in the Center for Translational Medicine.

Jurisdiction and Venue

6. This Court has subject matter jurisdiction over this matter pursuant to 28 U.S.C. § 1331, as this matter pertains to a federal question relating to misappropriation of trade secrets under 18 U.S.C. § 1831, et seq. This Court also has supplemental jurisdiction under 28 U.S.C. § 1367 over the Pennsylvania state law claims asserted herein.

7. Venue is proper in this Court under 28 U.S.C. § 1391(b) as it is the judicial district in which Defendants reside and is the location where a substantial part of the events and omissions giving rise to the claims asserted herein occurred.

Background Facts

Dr. Houser's Research and Reputation

8. Dr. Houser earned his PhD in Physiology, completed a research fellowship in the Division of Cardiology at the Lewis Katz School of Medicine at Temple University ("School of Medicine") and joined the Temple faculty as an Assistant Professor of Physiology in 1979.

9. Since joining Temple in 1979, Dr. Houser has become an internationally respected cardiovascular researcher who has been a faculty member of the School of Medicine and Temple itself for more than forty (40) years.

10. Under Dr. Houser's leadership, his research group has published approximately 240 professional articles and helped identify defective molecular and cellular processes that produce weak cardiac muscle cells that can cause poor heart function and lead to heart failure.

11. Dr. Houser's laboratory has been continuously funded by the National Institutes of Health ("NIH") for more than 30 years, and in 2012, Dr. Houser's group was awarded a five-year, $11.6 million grant from the National Heart, Lung and Blood Institute of the NIH to study approaches to prevent or reverse damage to the heart after a heart attack.

12. Over the course of his illustrious career, Dr. Houser rose through the ranks and was appointed as Director of the Lewis Katz School of Medicine ("School of Medicine") at Temple University Cardiovascular Research Center in 2003 (which he co-founded) and Chair of Physiology in 2005 (a position he continues to hold as of the filing of this Complaint). Also, he serves the School of Medicine as Senior Associate Dean of Research.

13. As a result of his efforts and those of other respected colleagues over the years, the School of Medicine has become one of the top research-oriented medical schools in the country and one of the top medical schools in Pennsylvania, and Dr. Houser was awarded an Endowed Chair ($2.5M), The Vera A Goodfriend Chair in Cardiovascular Sciences.

14. Dr. Houser's stellar reputation as an academician and researcher has been honed over many decades and thousands of hours of detailed and quality work.

15. Dr. Houser relies on his reputation, moreover, to help his group and Temple itself gain international attention, grants, speaking engagements and other special opportunities for him and the group that he has led for decades.

16. Further, as a researcher, Dr. Houser places the utmost importance on academic ethics and the integrity of his research and work. He has risen through the ranks of the major professional and service organization in his field, the American Heart Association ("AHA"). Dr.

Houser has served in numerous leadership roles in this organization, served on the Board and in 2017 served as the AHA President, the first Ph.D. basic scientist to hold this position.

The Employment Agreement and Temple Policies

17. Over the course of his decades at Temple, Dr. Houser has been a party to many agreements with Temple and has, over time, become a tenured member of Temple's faculty.

18. Successively year after year, Dr. Houser received exceptional performance reviews from Temple (and his students and colleagues), and from 1979 through the present, Temple offered Dr. Houser continued employment, raises, bonuses and ever-increasing responsibility.

19. Dr. Houser, in addition to his academic and research skills, has also proven himself to be an outstanding manager and administrator.

20. Accordingly, on March 20, 2012, Dr. Houser entered into an employment agreement to serve as the Chairperson of the Department of Physiology and Director of the Cardiovascular Research Center ("2012 Agreement"). A copy of the March 20, 2012 agreement is attached hereto as Exhibit "A."

21. The 2012 Agreement provided, among other things, that –

a. The term of the agreement ended on June 30, 2017;

b. Dr. Houser served at the "pleasure of the Dean"; and

c. Dr. Houser was responsible, both as Chairperson and Director, for "adhering to the policies and guidelines of the department, school, university, and accrediting bodies", "ensuring compliance with all regulations of the Center, School, university and external funding agencies, including time and effort reporting"

22. On March 23, 2017, Dr. Houser entered into another employment agreement ("2017 Agreement") with Temple. A copy of the March 2017 agreement is attached hereto as Exhibit "B."

23. Through the 2017 Agreement, Temple renewed Dr. Houser's appointments as Senior Associate Dean, Research, Chair of the Department of Physiology, and Director of the

Cardiovascular Research Center in the School of Medicine from January 1, 2017 to June 30, 2022.

24. In the 2017 Agreement, Temple states that Dr. Houser is responsible for "ensuring compliance with all regulations of the Center, School, University and external funding agencies, including time and effort reporting" and "Compliance with sponsor, University and School policies."

25. The 2012 Agreement and 2017 Agreement are sometimes hereafter collectively referred to as the "Employment Agreements."

26. The Employment Agreements incorporated Temple's various "policies and guidelines" as terms and conditions of Dr. Houser's employment and Temple's obligations to Dr. Houser.

27. Through the Employment Agreements, Temple placed significant responsibility and visibility in Dr. Houser.

28. Dr. Houser, accordingly, took his responsibilities seriously and undertook a program of the highest academic, ethical and research standards.

29. As part of Temple's policies and procedures which applied to the Employment Agreements, the Board of Trustees issued the Policy on Misconduct in Research and Creative Work, Policy No. 02.54.01 (the "Creative Work Policy") with an effective date of May 14, 2002.

30. Through the Creative Work Policy, Temple covenanted to Dr. Houser, among others, that it was "committed to generating and disseminating knowledge and to protecting traditional principles of academic freedom." *See* Creative Work Policy at Art. I.

31. Temple further acknowledged that it "recognizes the importance of protecting the lives and rights of all who are involved in those processes and of maintaining a relationship of trust within the broader academic, research and social communities." *Id.*

32. Temple mandated "that each person who engages in or supervises research or creative work be responsible for conducting these activities in an ethical manner." *Id.*

33. Temple stated that the Creative Work Policy applies to "all faculty members." *Id.* at Art. III.

34. The Creative Work Policy provides for different procedures for tenured (Group 1) and untenured faculty (Group 2). *See id.* at Art. II. n.1.

35. In the Creative Work Policy, the process for Group 1 (or tenured faculty) begins "[u]pon receiving information that a violation of the policy is alleged to have occurred[.]" *Id.* at Art. II.A.1.

36. Upon receiving such information, however, Temple promised that "the Integrity Officer preliminarily will assess whether sufficient information exists to refer the matter to an Inquiry Committee." *Id.*

37. The Creative Work Policy does not authorize the Integrity Officer to conduct an investigation as part of any preliminary assessment. *See generally* Creative Work Policy.

38. For Group 1 personnel, Temple agreed that the Inquiry Committee "will be the Faculty Senate Personnel Committee ('Personnel Committee')." *Id.*

39. In point of fact, under the Creative Work Policy, if the Integrity Officer "reports the matter to the Personnel Committee, the Personnel Committee will determine (1) whether there is sufficient evidence of actionable misconduct to warrant an Investigation, and (2) if so, whether in its view, formal proceedings to consider dismissal or discipline should be instituted." *Id.* at Art. II.A.2.

40. Thereafter, the President of Temple, "after reviewing the findings and recommendation of the Personnel Committee, will decide whether to institute formal proceedings for discipline up to and including dismissal." *Id.* at Art. II.A.3.

41. Temple guaranteed Group 1 personnel that "If the President institutes formal proceedings, the Personnel Committee will appoint an Investigation Committee (Hearing Committee) to determine whether actionable misconduct occurred and to recommend appropriate action to the President." *Id.* at Art. II.A.4.

42. Finally, the "President or the President's Designee will decide what action(s) to take." *Id.* at Art. II.A.5.

43. The process provided by the Policy for initiating an Inquiry is as follows: "If an allegation of misconduct proceeds to an Inquiry, the Integrity Officer promptly will (1) notify the Respondent, in writing, that an Inquiry Committee will be convened to consider an allegation of misconduct, (2) specify the nature of the allegation, (3) provide a copy of this policy to the Respondent, and (4) notify the Respondent that he or she will have the opportunity to be interviewed by and present evidence to the Inquiry Committee." *Id.* at Procedures, Art. I.B.1.

44. Most significantly, Temple promised that "Inquiries" must "be completed within 60 and 90 days…from the date of their initiation" and "Investigations … must be completed within 120 and 180 days…from the date of their initiation." *Id.* at Policy, sec. J.

45. According to the Creative Work Policy, "the Integrity Officer may approve an extension of these periods, in writing, if circumstances clearly warrant a longer period. The Respondent will be notified of the extension and the reason for the extension. The reason for the extension will be stated in the Inquiry Report." *Id.* at Policy, sec. F.

46. Temple promised that all "Inquiries and Investigations will be conducted in a manner that will ensure fair treatment and confidentiality to the Respondent to the maximum extent reasonably possible without compromising public health and safety or thoroughness in carrying out the Inquiry or Investigation." *Id.* at Policy, sec. D.

47. The Creative Work Policy requires all faculty members to cooperate with the Integrity Officer but does not require a Respondent "to provide written or oral testimony." *Id.* at Policy, sec. E.

48. Temple further promises that "confidential treatment will be afforded to all affected, to the maximum extent reasonably possible." *Id.* at Policy, sec. F.

49. The Creative Work Policy also defined various elements presumably to provide clarification to the process:

a. Allegation, according to Temple, "means any written or oral statement or other indication of possible misconduct, as defined in this policy, clearly communicated to the Integrity Officer." *Id.* at Art. III.A.

b. Good faith, according to Temple, "means the honest belief that misconduct may have occurred. An allegation is not made in good faith if it is made with reckless disregard for or in willful ignorance of facts that would disprove the allegation." *Id.* at Art. III.D.

c. Integrity Officer, according to Temple, "means the University's chief research officer, as designated by the President." *Id.* at Art. III.I.

d. Misconduct, according to Temple, "means fabrication, falsification, plagiarism, or other practices that seriously deviate from those that are commonly accepted within the research and creative communities for proposing, conducting, or reporting research or other creative work." *Id.* at Art. III.L.

i. Temple further defined "Misconduct", in relevant part, as follows:

1. Abuse of confidentiality: taking or releasing the ideas or data of others that were shared with the legitimate expectation of confidentiality; *e.g.*, stealing or disseminating ideas from others' grant proposals, award applications or manuscripts for publication when one is a reviewer for granting agencies or journals. *Id.* at Art. III.L.1.

2. Dishonesty in publication: knowingly publishing material that will mislead readers, e.g., misrepresenting date or their origin, misrepresenting research progress or adding or deleting the names of other authors without their permission. *Id.* at Art. III.L.5.

3. Plagiarism: taking credit for someone else's work and ideas, stealing others' results or methods, copying the writing of others without proper acknowledgment, or otherwise taking credit for the work or ideas of others. *Id.* at Art. III.L.9,

> 4. Property violations: misappropriating, stealing or destroying equipment, supplies or other information including, but not limited to, data, text, works of art or authorship or databases, which either belong to others or over which others have primary usership. *Id.* at Art. III.L.10.

50. Thus, under the Creative Work Policy, an "Inquiry" or an "Investigation" are the fact-finding processes authorized by Temple and agreed to by the faculty.

Temple's Intellectual Property Policies

51. Temple also has policies respecting "Inventions and Patents" (hereafter, "Inventions Policy").

52. Temple's Inventions Policy, the current iteration of which was adopted by Temple's Board of Trustees in 1985, provides that the school "encourages the search for new knowledge, discoveries and inventions which will serve the public good." *See* Inventions Policy at ¶ 1.

53. Temple has stated that this policy is intended to "provide incentive for those engaged in research and the applied arts to commercialize their inventions" and to "define the obligations of all parties involved in the invention process and to safeguard their rights and interests." *Id.* at ¶¶ 1(a) and (b).

54. The Inventions Policy provides for the assignment of all rights and interest in any invention developed in whole or in part by any university employee with any university facilities or equipment to Temple itself. *Id.* at ¶ 2.

55. Where an invention has been identified, Temple has, among others, the obligation to determine the owners of any such invention by reference of the matter to the president or person designated by the president to do so. *Id.* at ¶¶ 2 and 3(f)(b).

56. Temple promised under the Inventions Policy that the "net income to the university from a royalty agreement or other agreement resulting from an invention shall be distributed as follows: 50% to the inventor(s) and 50% to the university, provided that the

inventor(s)' share shall not fall below five percent (5%) of the university's gross receipts." *Id.* at ¶ 4.

Feldman and the Misappropriation of Dr. Houser's Research

57. Feldman was hired as Executive Dean of the School of Medicine in or around 2012 or 2013.

58. Dr. Houser reported directly to Feldman in affairs regarding Dr. Houser's positions with Temple until Dr. Feldman was relieved of his duties by Temple as Executive Dean on or about December 21, 2015.

59. In 2015, Dr. Houser and his lab at the School of Medicine were working on many projects related to heart failure. As part of one of those projects, Dr. Houser and his lab conducted experiments to examine changes in cardiac structure and function after an induced "myocardial infarction," commonly known as a heart attack.

60. As part of this project, the lab had developed a large animal (in this case, a pig) model to study heart failure after a myocardial infarction. Large animal models, such as a pig model, are an important precursor to the eventual development of treatments and therapies for humans as they can more closely mimic and predict conditions that are likely to occur in humans.

61. Dr. Houser's lab was using this pig model to test novel cell and drug therapies for the treatment of the failing heart. As a result of this testing and research, Dr. Houser's lab developed a pig model that shows, among other things, what happens to the heart of a patient who has suffered a heart attack (via a blocked coronary artery), is rushed to the hospital and has their blocked artery reopened after ninety (90) minutes. In other words, Dr. Houser's pig model establishes a model of the "gold standard" of time between when a heart attack occurs and when treatment is rendered in a typical human patient (hereafter, "Dr. Houser's Pig Model").

62. As part of the research, Dr. Houser's team performed testing that showed that the subjects suffered heart failure with reduced ejection fraction (a decrease in percentage of blood being pumped from the heart with each heartbeat) after inducing a myocardial infarction (heart attack) (hereafter, "Dr. Houser's Pig Data").

63. Dr. Houser's Pig Data was part of a broader study in the Houser lab and were not published at the time. This work was initially funded by Dr. Houser's endowed chair and later was funded by the NIH. Dr. Houser's Pig Data was not public nor was it meant to leave Dr. Houser's lab without his express permission.

64. At or about the same time that Dr. Houser was working on research related to his Pig Model, Feldman was working on a project to see if a molecule, known as "BAG3," was altered in heart failure and if correcting the defective molecular concentration would rescue the heart function.

65. Heart failure in humans comes in two general forms, divided by whether or not "ejection fraction" is reduced. Ejection fraction, most simply, is the percentage of blood that is ejected from the heart with each heartbeat.

66. Heart failure with reduced ejection fraction has multiple causes, but the most prevalent is "ischemic heart disease" or heart disease that results from a blockage of blood flow (such as from atherosclerosis), which leads to myocardial infarction (heart attack). After a person has a myocardial infarction, they have a dead region of their heart which causes the heart to remodel and eventually its ability to pump blood is reduced (*i.e.*, the ejection fraction is decreased). Most clinical research has involved this form of heart failure.

67. The other major form of heart failure is heart disease in which ejection fraction is not reduced. This type of heart failure can result from, among other causes, hypertension (high blood pressure).

68. Upon information and belief, Feldman's work on BAG3 consisted of (1) research related to a small population of human patients suffering from heart failure, with genetic mutations that cause reduced levels of BAG3; and (2) a mouse model that induces heart failure through hypertension, or pressure overload, in the heart, and that has reduced levels of BAG3.

69. Upon information and belief, Feldman observed that patients with a rare hereditary form of heart failure (familial dilated cardiomyopathy), resulted from a mutation in the BAG3 gene.

70. Upon information and belief, Feldman also observed that in a mouse model, where heart failure was induced as a result of pressure overload of the heart, BAG3 levels were reduced. The mouse model, however, lacks broad clinical significance, as the acute nature of the pressure overload induced in the mouse in order to cause heart failure, does not occur in humans. In other words, the mouse model does not mimic what happens when a patient suffers a heart attack. A heart attack results from ischemic heart disease, a blockage of blood flow in a coronary artery, not from an overload of pressure caused by constriction of an artery.

71. Upon information and belief, Feldman did not have a pig model as part of his BAG3 research. It was important for Dr. Feldman to have access to a large animal model, especially one that mimicked the conditions of a patient suffering a heart attack—ischemic heart disease (heart disease that results from a blockage of blood flow) with reduced ejection fraction (the amount of blood that leaves the heart each time it contracts). Such a model would have broad clinical significance as it would allow for the testing of potential treatments and therapies for human patients who have suffered a heart attack, the leading cause of ischemic heart failure in humans.

72. Upon information and belief, having such a model also was important to secure investment and other financial support for a commercial venture based upon this invention.

73. Thus, using data and testing from a large animal model, such as Dr. Houser's Pig Model, was an essential part of the inventive and commercialization processes to complete Dr. Feldman's research. If Dr. Houser's Pig Model, which mimics the 90-minute gold standard of the time between the occlusion event (blocked artery) and reperfusion (what occurs when a patient suffers a heart attack, is rushed to the hospital, and the blocked artery is reopened), also showed reduced levels of BAG3 in the heart tissue, the model could be used to test the potential efficacy of gene therapies that would reintroduce BAG3 to the damaged tissue in the hopes of recovering normal heart function, including improving the heart's ejection fraction (ability to pump blood).

74. At the time, Dr. Feldman and Dr. Houser were generally aware of each other's work; however, Dr. Houser was not aware that Dr. Feldman needed access to Dr. Houser's Pig Model for any future commercial endeavors. As Dr. Houser's supervisor, however, Dr. Feldman was permitted to be and was aware of the significance and some details of Dr. Houser's research and of the potential value of Dr. Houser's Pig Model.

75. Although Dr. Feldman was permitted to be aware of Dr. Houser's research due to his role as Dr. Houser's supervisor, he was not, however, permitted to use this research without Dr. Houser's permission and, even more egregiously, claim it as his own. Indeed, in so doing, Dr. Feldman violated not only Dr. Houser's legal rights but also the fundamental principles of academic integrity and integrity of scholarship.

76. Upon information and belief, with this knowledge, Dr. Feldman seized on the opportunity to secure Dr. Houser's Pig Model without investing the time, money and research necessary to create one for himself. Thus, Dr. Feldman embarked on a covert effort to improperly secure access to this pivotal missing link for his research, without having to share the credit (or potential financial gains) from this invention with Dr. Houser.

77. Upon information and belief, in or about 2014 or 2015, Dr. Feldman, while he was Dr. Houser's supervisor and Executive Dean of the School of Medicine, approached one of Dr. Houser's graduate students, Thomas Sharp, who had worked on the Pig Model with Dr. Houser, and asked Sharp to prepare several figures with "hemodynamic" data (*i.e.*, cardiac structure and function data, from Dr. Houser's Pig Data that Dr. Houser had developed in his lab. These figures became incorporated in both the paper and the patent applications discussed below.

78. Dr. Houser's Pig Data was maintained on Sharp's Temple provided computer in Dr. Houser's lab under password protection. A back up of Dr. Houser's Pig Data was also maintained on a portable hard drive that is kept locked in Dr. Houser's office. Only Sharp and Dr. Houser had access to this data.

79. Dr. Houser's Pig Data is further secured from tampering because Dr. Houser's lab follows coding and data security protocols recommended by NIH to reduce bias and enhance the reliability of the data. Among other things, all data is coded so as to not visibly link it with a particular animal during the analysis. The coded records are separately maintained by the manager of Dr. Houser's lab in a locked and secure manner.

80. Feldman also asked Sharp for samples of tissue from pigs that had undergone the 90-minute occlusion to induce a heart attack and control samples ("Dr. Houser's Pig Model Tissue Samples").

81. Dr. Houser's Pig Model Tissue Samples were kept in a secured -80 degree freezer in the Cardiovascular Center (10th Floor of the Medical Education and Research Building) to which only Dr. Houser, Sharp and other members of Dr. Houser's lab who supported his work including on the Pig Model, had access. The -80 freezer is identified as belonging to Dr. Houser's Lab only. Within the freezer, all tissue samples are stored in coded boxes. Only members of Dr. Houser's Lab were privy to the coding that indicated the nature of the samples.

82. Upon information and belief, Feldman used Dr. Houser's Pig Model Tissues Samples in his lab to measure the BAG3 protein in the samples, the results of which were also incorporated into the paper and the patent applications discussed below.

83. Upon information and belief, Dr. Feldman told Sharp that (1) Dr. Houser had given his permission for Sharp to provide Dr. Houser's Pig Data and Dr. Houser's Pig Model Tissue Samples to Dr. Feldman; (2) Dr. Houser would be able to examine the paper which Dr. Feldman was working on before submission; and (3) Dr. Houser would be identified as a co-author on the paper. The first of these statements is categorically false. Upon information and belief, the remaining two statements were also false when made.

84. Upon information and belief, Dr. Feldman intentionally and deliberately misled Sharp into preparing summary figures from Dr. Houser's Pig Data for Dr. Feldman's use and providing Dr. Houser's Pig Model Tissue Samples to Dr. Feldman both by claiming that Dr. Houser had authorized such activities and by using his leadership position within the University to lend authority to this request.

85. In fact, Dr. Houser had no knowledge of Dr. Feldman's request to Sharp, nor did he give Dr. Feldman permission to take, use, publish or otherwise claim as his own Dr. Houser's Pig Data, Dr. Houser's Pig Model Tissue Samples or any data derived from Dr. Houser's Pig Model Tissue Samples. Indeed, Dr. Feldman never made any such requests for permission to Dr. Houser.

86. Unbeknownst to Dr. Houser, Sharp (who, upon information and belief, believed he was acting with Dr. Houser's knowledge and blessing) gave the figures created from Dr. Houser's Pig Data and Dr. Houser's Pig Model Tissue Samples to Dr. Feldman (hereafter, Dr. Houser's Pig Data and Dr. Houser's Pig Model Tissue Samples will be referred to collectively as the "Stolen IP").

87. Unbeknownst to Dr. Houser, Dr. Feldman published a paper which contained the Stolen IP and data derived therefrom in or around 2015 (hereafter, the "Feldman Paper"). A copy of the Feldman Paper is attached hereto as Exhibit "C."

88. Dr. Houser, however, was never provided with a copy of the Feldman Paper at any time prior to its submission (and only learned of it until years after it was first published).

89. Dr. Houser was not listed as a co-author on the Feldman Paper or any other paper, article or any other document that used the Stolen IP, Dr. Houser's Pig Data or any information derived from Dr. Houser's Pig Model Tissue Samples.

90. Further, while Dr. Feldman intentionally omitted Dr. Houser as a co-author on the Feldman Paper, he listed Sharp, Dr. Houser's graduate student, as a co-author. In such situations, it is customary for the head of lab (Dr. Houser, in this case) to be listed, and the head of the lab communicate to the other lab director (in this case, Dr. Feldman), who had performed sufficient work to be listed as a co-author. That Dr. Houser was never contacted by Dr. Feldman about either the Stolen IP or the Feldman Paper, but his graduate student was (and even listed as a co-author), underscores that Dr. Feldman intentionally excluded Dr. Houser from the entire process. Upon information and belief, Dr. Feldman also never informed Sharp that Dr. Houser was not being listed as a co-author and Sharp also did not learn of this until years after the Feldman Paper was published.

91. Sometime in 2017, Dr. Houser learned from Sharp, that at some point in either 2014 or 2015, Dr. Feldman had requested the Stolen IP, told Sharp that Dr. Houser had granted permission to Dr. Feldman to use the Stolen IP and stated that he would give Dr. Houser co-author credit on a paper which Feldman would publish.

92. At or about the same time in 2017, Dr. Houser first learned of the Feldman Paper published by Dr. Feldman, which relied, critically, on Dr. Houser's Pig Data as well as data

derived from Dr. Houser's Pig Model Tissue and passed off the research, data and model as if it were Dr. Feldman's own research, model and data and not that of Dr. Houser.

93. Dr. Feldman used the Stolen IP and data derived therefrom in the Feldman Paper. Specifically, in the paper, Dr. Feldman published figures derived from Dr. Houser's Pig Data to show that the subjects suffered heart failure with reduced ejection fraction (the decrease in blood flow being pumped through the heart that occurs during a heart attack), and the results of protein testing done on Dr. Houser's Pig Model Tissue that showed reduced levels of BAG3 in the heart tissue from the pig where a heart attack was induced.

94. The Stolen IP provided necessary clinical information needed by Dr. Feldman to begin to market BAG3 as a potential therapeutic target for patients who suffer from a heart attack, the leading cause of ischemic heart failure (heart failure caused by a blockage of blood flow through the heart).

95. The Feldman Paper focused on the available data and evidence supporting the theory that BAG3 may be a potential target for new therapies in patients with heart failure.

96. Since Dr. Feldman published the Stolen IP as Dr. Feldman's own work, Dr. Houser is unable to claim the Stolen IP as his own work in his own papers, grant reports or new grant proposals.

97. In 2017 when he learned of Dr. Feldman's misconduct, Dr. Houser knew that he, as the author/creator of the Stolen IP, had not given Feldman permission to use the Stolen IP or permission to pass off the Stolen IP as Feldman's own work.

98. Dr. Houser also knew that he, as the author/creator of the Stolen IP, had not been approached or contacted by Dr. Feldman or anyone on his behalf to use the Stolen IP in the Feldman Paper or otherwise.

99. Dr. Feldman's conduct constituted misconduct under Temple's own Creative Work Policy, including dishonesty in publication, plagiarism and property violation. *See* Art. III.L(5), (9) and (10).

100. In accordance with Temple's Creative Work Policy, in or around February 2017, Dr. Houser made a good faith allegation of misconduct by Dr. Feldman to Dr. Susan Wiegers who was at that time the Senior Associate Dean of Faculty Affairs at the School of Medicine.

101. Upon information and belief, Dr. Wiegers turned over Dr. Houser's report to the University's Integrity Officer and Vice President of Research ("OVPR") at Temple, Michele Masucci ("Masucci").

102. Dr. Houser was never interviewed over the matter by the OVPR.

103. Dr. Houser was never notified whether there was an Inquiry or Investigation, as those concepts are defined in Temple's policies, over Dr. Feldman's conduct.

104. Instead, approximately one week after Dr. Houser's report, Dr. Wiegers told Dr. Houser that the OVPR would like him to accept an "apology" from Dr. Feldman.

105. Dr. Feldman offered an "apology" to Dr. Houser but did not return any part of the Stolen IP, and the Feldman Paper was not corrected to reflect Dr. Houser as an actual author/creator of the lab data upon which the paper (and Dr. Feldman) so critically relied.

106. Dr. Houser believed he had no further recourse regarding the Stolen IP given that Temple's solution to his complaint was to have Dr. Feldman apologize. In 2017, having reported the issue to the University, Dr. Houser also did not believe that any further uses would be made of the Stolen IP and he considered the matter resolved. Specifically, under no circumstances could Dr. Houser have anticipated that, having become aware of the true owner of the Stolen IP, Temple would permit Dr. Feldman to apply for or pursue patent applications using the Stolen IP, knowing that it consisted of or was derived from Dr. Houser's work and not Dr. Feldman's.

107. To this day, Dr. Houser has not given Dr. Feldman any permission to use, publish or otherwise claim the Stolen IP as Dr. Feldman's own in any forum or publications.

108. In December of 2015, Dr. Feldman was relieved by Temple of his position as Executive Dean but remained on staff at Temple and the School of Medicine.

109. Dr. Houser no longer reported directly to Dr. Feldman after that point.

110. Despite being colleagues at Temple, Dr. Houser understands that Dr. Feldman dislikes Dr. Houser, and upon information and belief, Dr. Feldman stands to lose considerable credibility and financial gain if his improper actions regarding Dr. Houser's Stolen IP come to light.

The So-Called Inquiry Or Investigation

111. In October 2018, Dr. Houser (and many other scientists and academics across the United States) was notified that Harvard University ("Harvard") was recommending the retraction of approximately 30 academic papers which were authored, co-authored or collaborated on by a now disgraced and former Harvard professor, Piero Anversa ("Anversa"), and/or his group (that is, Anversa, plus a number of other faculty who joined Harvard from New York Medical College when Anversa did).

112. As a prominent academic and scientist, Anversa (and his group) had collaborated with a large number of people and published papers with many more.

113. Harvard, however, focused its investigation on papers published by Anversa and his group while they were at Harvard.

114. Dr. Houser had one research paper that was named in the Harvard investigation; however, Anversa was **neither** an author **nor** a co-author on that paper.

115. Houser and Anversa have no co-authored papers during Anversa's tenure at Harvard.

116. The research paper, entitled *"Increasing Cardiac Contractility After Myocardial Infarction Exacerbates Cardiac Injury and Pump Dysfunction."* Authored and co-authored by: Hongyu Zhang, Xiongwen Chen, Erhe Gao, Scott M. MacDonnell, Wei Wang, Mikhail Kolpakov, Hiroyuki Nakayama, Xiaoying Zhang, Naser Jaleel, David M. Harris, Yingxin Li, Mingxin Tang, Remus Berretta, Annarosa Leri, Jan Kajstura, Abdelkarirn Sabri, Walter J. Koch, Jeffery D. Molkentin, Steven R. Houser.: *Circ. Res.* 2010;107: 800-809. (hereafter, the "2010 Paper"), was published by AHA after peer review.

117. Peer review is a critical component of the academic verification and vetting process for research papers. In peer review, the authors send the paper to a journal. The journal then sends the paper to three experts in the particular field at issue. The authors do not know (and they do not choose) the reviewers, and the reviewers remain anonymous to the authors through the peer review process. The three selected experts critique the paper (nothing is off limits in the review process), and for good journals about 80% of all submissions are rejected based on peer review. If the paper is deemed suitable for publication, the authors are given the opportunity to answer questions, provide more data, and revise the paper as needed. The peer review process can go back and forth a number of times before the paper is finally accepted for publication.

118. A leading hypothesis Anversa was known for was the idea that a "stem" cell exists in the heart which could regenerate new cardiac muscle cells called "myocytes."

119. The 2010 Paper, however, did not focus on this theory and instead concerned myocyte (or muscle cell) death from too much calcium.

120. Anversa was neither a collaborator or co-author on the 2010 Paper.

121. Instead, Harvard expressed concern that others at Harvard and members of Anversa's "group" – but not Houser – might have created (or helped to create) a fabricated supplemental figure included in the 2010 Paper.

122. Upon learning of the potentially-fabricated figure, Dr. Houser, along with his primary collaborator Jeffrey Molkentin, PhD, from Cincinnati Children's Hospital, requested that the publisher, AHA, allow them the opportunity to correct the figure, which was not central to the conclusions of the paper.

123. AHA agreed to permit the corrected figure, and Drs. Houser and Molkentin later provided data to support the original paper and a ***new*** figure to AHA for correction of the 2010 Paper. AHA accepted the correction and the Notice of Concern, issued while the suspect figure remained uncorrected, was removed. The 2010 Paper otherwise had become and remains accepted and competent scientific research in the field.

124. Moreover, for any paper where Anversa is identified as a senior author while at Harvard, there is no data from Dr. Houser's lab at the School of Medicine.

125. Nevertheless, not long after Harvard's announcement about Anversa, from around October 2018 and through September 2019, Masucci spoke multiple times to Dr. Houser, stating that she would need to begin an "inquiry" – presumably in accordance with the Creative Work Policy – related to the 2010 Paper as a result of Harvard's call for retraction of papers involving Anversa or his group. Harvard made no claims about any individuals other than Anversa and other Harvard faculty in his research group.

126. However, the real reasons for the Inquiry were not disclosed to Dr. Houser.

127. Masucci told Dr. Houser, moreover, that due to her friendship with him, she had a conflict of interest in any inquiry or investigation respecting Dr. Houser, and therefore Michael Henderson, an attorney in the OVPR office, would be in charge of the process. For all purposes related to this alleged Inquiry/Investigation, it appeared to Dr. Houser that Henderson was merely following Masucci's instructions.

128. Henderson left Temple about a year ago, and for all relevant times hereafter, Masucci has taken over the Inquiry/Investigation at issue, despite her claims of an alleged conflict of interest.

129. In the midst of the discussions between Masucci and Dr. Houser, however, Dr. Houser received an email providing him with the time and place at which he had to meet with attorneys from Wilmer Hale, an outside law firm presumably retained by Temple to conduct an investigation into the Anversa matter, and to be prepared to discuss the 2010 Paper with the attorneys.

130. On October 10, 2019, Dr. Houser met with three attorneys from Wilmer Hale and was questioned for approximately five hours.

131. The first question from the attorneys handling the Inquiry/Investigation concerned the reason why Anversa was not identified as a co-author on the 2010 Paper.

132. Dr. Houser explained that the reason he was not so identified was that he did not do anything on or relating to the 2010 Paper.

133. Though the email Dr. Houser received stated that he should be prepared to discuss the 2010 Paper, most of the interrogation focused on other matters not mentioned to Dr. Houser before, including a paper published in 2007 from Dr. Houser's lab in which Anversa was identified as a collaborator (hereafter, the "2007 Paper").

134. In 2007, Anversa was a faculty member at New York Medical College and not Harvard.

135. To Dr. Houser's knowledge at the time of his interrogation by Temple's outside lawyers engaged to conduct an investigation, there had been no claim of fraud, fabrication, plagiarism, or academic misconduct of any kind leveled against the 2007 Paper.

136. At the 2019 interrogation, Dr. Houser had no counsel or representative present with him nor was he advised at any point before, during or after the interrogation of any basis for any Inquiry or Investigation by Temple.

137. There is no authorization under the Creative Work Policy for the Integrity Officer or Temple or anyone acting on their behalf to require an interrogation or document production pursuant to a "preliminary assessment." *See generally* Creative Work Policy.

138. Dr. Houser nevertheless answered all questions posed by the Wilmer Hale attorneys, despite having no obligation to do so.

139. After Harvard's listing of retraction requests in October 2018 were made public, Dr. Houser was informed by certain colleagues that Dr. Feldman had told them that Dr. Houser was under investigation by Harvard and the National Institutes of Health ("NIH").

140. Dr. Houser, in fact, informed Masucci that Dr. Feldman was making these untrue statements about him to other faculty members.

141. In point of fact, Dr. Houser was asked on separate occasions by Dr. John Daly (formerly the interim Dean of the School of Medicine) and Patrick O'Connor (at the time the Chairman of the Temple Board) to explain the so-called "Harvard Investigation."

142. As an aside, Dr. Houser also informed the Wilmer Hale interrogators that Dr. John Daly and Patrick O'Connor had approached him to inquire about rumors they heard which were the same as those being disseminated by Dr. Feldman.

143. The statements that Dr. Houser was being investigated by Harvard and/or NIH are categorically false.

144. The same statements were intended to defame Dr. Houser and harm his reputation and personal and professional standing in his profession.

145. To Dr. Houser's knowledge, Temple has not initiated any inquiry or investigation into Dr. Feldman's defamatory statements against him.

146. On October 10, 2019, in the midst of this non-specific "Inquiry/Investigation", Dr. Houser contacted Masucci to ask whether the Stolen IP which Dr. Feldman wrongfully claimed as his own was used in any patent filings.

147. By that time, Dr. Houser knew that Dr. Feldman had used the Stolen IP without Dr. Houser's permission in the Feldman Paper and wondered if the Stolen IP might also be part of a patent filing.

148. In response to Dr. Houser's questions, Masucci stated that she did not know the answer but noted that "it is a fair question." Masucci next stated that "I would like to move through our current process and then take a step back to assess how best to address many interrelated matters."

149. In January 2020, Dr. Houser, frustrated with the lack of information and responsiveness to his questions about the Stolen IP and Dr. Feldman's defamatory statements about him, met with counsel for Temple, Michael Gebhardt, to discuss the lies Dr. Feldman had been spreading about Dr. Houser, as well as Dr. Houser's concerns regarding Dr. Feldman's use of the Stolen IP.

150. Thus, Temple, at the highest levels, was aware of Dr. Houser's concerns and the reports of Dr. Feldman's conduct.

151. To date, however, neither Masucci nor Temple ever responded to Dr. Houser's inquiries about these matters in any substantive way.

152. In the meantime, Dr. Houser's direct reports and other colleagues of his in the School of Medicine were being interviewed by either Temple or its counsel, Wilmer Hale, in connection with the unspecified claims against him.

153. In point of fact, on August 31, 2020, Masucci requested that a co-worker of Dr. Houser "gather[] information related to the collaboration between the laboratory of Dr. Steven Houser and Dr. Piero Anversa" and demanded that Dr. Houser's colleague "collect your

laboratory notebooks." Masucci told Dr. Houser's co-worker that "Dr. Houser is aware that we have requested the preservation of documents related to this matter; however, we request that you keep this matter confidential and ask that you do not discuss our request or share this message with your colleagues," which obviously included Dr. Houser.

154. Dr. Houser was unaware of Masucci's request sent to his colleague when the request was made.

155. Further, Masucci was told by Dr. Houser's co-worker who was the target of Masucci's document demands that when the notes were delivered to her office, the notes at issue were really Dr. Houser's property and they were stored in his private storage areas. Dr. Houser's co-worker expressed concern about continuing to act upon Masucci's requests.

156. Masucci responded that "we will take care of it from here."

157. Masucci also made a request for lab notebooks of a former trainee in Dr. Houser's lab from another co-worker, again all without Dr. Houser's knowledge or consent.

158. Dr. Houser, not initially knowing about Temple's secretive efforts to collect his lab work products, reported a theft of property from his lab to the police.

159. To date, there exists a gap in Dr. Houser's lab notes and data from 2003-2013, as a result of documents demanded by and apparently given to Masucci as part of the Inquiry/Investigation against Dr. Houser.

160. After October 2019 and through August, 2020, Dr. Houser made several requests, at least once through his own legal counsel, for Masucci and/or Temple to identify the specific issues for the "Inquiry" or "Investigation" that they were pursuing against him.

161. Finally, on September 25, 2020, Masucci sent Dr. Houser an email in which she, for the first time, attempted to classify the nearly year-long inquiry as a "preliminary assessment."

162. Masucci said that "scope of the preliminary assessment from the outset involved your publications and research performed in collaboration with Dr. Anversa." Masucci also stated that the "preliminary assessment continues to focus on issues related to [certain publications relating to Anversa] and research performed in collaboration with Dr. Anversa."

163. Masucci then stated:

> Notwithstanding our efforts, we have not been able to review certain potentially relevant sources of information with respect to the collaboration. Thus, we are continuing to identify additional relevant sources of information to aid our assessment. Our expectation is that counsel will be prepared to sit with you for a final interview within the next 45 days, after which we will conclude the preliminary assessment.

164. Masucci's statement was false to the extent it tried to paint the ongoing "Inquiry" as a "preliminary assessment."

165. Nothing in the Creative Work Policy permits such a wide-ranging investigation to constitute a "preliminary assessment." *See generally* Creative Work Policy.

166. In fact, Masucci was conducting an "Inquiry" or "Investigation," which is not authorized by the Creative Work Policy to be conducted in the manner it has proceeded against Dr. Houser.

167. Dr. Houser provided a detailed written response, repudiating Masucci's mislabeling of Temple's actions and giving an answer to every allegation raised by Masucci.

168. In turn, Masucci responded stating that "this matter is not an 'inquiry' into misconduct, but rather is a 'preliminary assessment' under our policy on misconduct in research." Masucci next informed Dr. Houser that because Temple deems the process a "preliminary assessment," Dr. Houser's request "to be apprised of the nature of an allegation against you is applicable to an Inquiry under our policy," not a preliminary assessment. Further, "[i]f this matter moves to an Inquiry, we would then share the specific allegations regarding potential research misconduct that would warrant that next step."

169. Further, Masucci stated that "at this stage, there is no allegation of research misconduct – only an assessment being undertaken to determine if anything rises to that level."

170. Masucci then demanded to know whether Dr. Houser would – again – be "willing to meet with the team" to interrogate him once more.

171. The Creative Work Policy does not require any faculty member or employee to submit to interrogation or document demands under any alleged "preliminary assessment." *See generally* Creative Work Policy.

172. Also by email dated September 25, 2020, Masucci sent Dr. Houser correspondence stating that Temple had been notified by the Office of Research Integrity of the Office of the Assistant Secretary for Health – U.S. Department of Health and Human Services (hereafter, "ORI") "that you have been named in a complaint regarding allegations of possible research misconduct stemming from but not limited to 'Clare Francis' complaints that were received by ORI, including but not limited to those noted here: https://pubpeer.com/search?q=steven+houser [hereafter, the "PubPeer Claims"]."

173. Masucci warned Dr. Houser "not to discuss the matter with colleagues" and to preserve and maintain "any and all records regarding your research[.]"

174. "Clare Francis" is a pseudonym used to describe unverified and anonymous complaints or allegations by unspecified individuals regarding claimed cases of plagiarism or fabricated or duplicated figures appearing in scientific journals and papers. It is an academic pseudonym for an internet "troll."

175. PubPeer's own disclaimer states that "The success of the site is due to the expertise and diligence of our users, who create all of its content. Nevertheless, comments should always be considered as sources of potentially useful information whose veracity readers must evaluate for themselves."

176. PubPeer also states that it "does not review comments scientifically and provides no warranty as to their veracity."

177. Thus, PubPeer is an anonymous, open forum where reviewers criticize publications without peer review, fact checking or substantiation.

178. Thereafter, Dr. Houser heard nothing more from Temple or Masucci on the PubPeer Claims until January 2021.

179. By email dated December 11, 2020, Masucci again notified Dr. Houser that Temple's outside interrogators, Wilmer Hale, were "prepared to sit with you for a final decision to further inform the conclusion of their assessment."

180. Dr. Houser, not being given adequate or proper explanations of the basis or claims for the Inquiry/Investigation, did not agree to Masucci's invitation to be interrogated – again – as there exists no basis under the Creative Work Policy for the procedure presented.

181. To date, Dr. Houser has not been given any further information on the Inquiry/Investigation that presumably related to the 2010 Paper, as detailed above.

182. By letter dated January 11, 2021, Masucci wrote in follow up to her prior "notice" of the PubPeer Claims and listed 9 different papers – one of which, published in various academic journals on a variety of topics, which were identified at PubPeer (hereafter, the "PubPeer Demand Letter").

183. Masucci's PubPeer Demand Letter does not identify any basis for any allegations other than the reference to PubPeer.

184. In point of fact, cross-referencing the list of 9 articles identified by Masucci in her PubPeer Demand Letter on PubPeer's own site confirms that comments on most of the claimed, anonymous criticisms appeared ***more than 6 months ago*** and one alleged comment specifically contains an Errata entry from one of the authors showing that the article was ***corrected*** long before Masucci's baseless PubPeer Demand Letter.

185. None of the PubPeer Claims, however, contain "strong evidence," as PubPeer requires, to support any of the allegations Temple now asserts.

186. Moreover, Masucci did not explain how or why these papers, which have been identified on PubPeer for months and corrected in one case, are now being raised against Dr. Houser.

187. Nevertheless, in her PubPeer Demand Letter Masucci demanded that Dr. Houser preserve documents and warned any failure to do so might constitute misconduct.

188. The PubPeer Demand Letter, however, did not mention the status of documents Masucci previously demanded and received from Dr. Houser's lab without his knowledge or permission.

189. Masucci also notified Dr. Houser that "Temple University will identify a time to hold an initial discussion of the specific allegations once the records you provide have been reviewed."

190. The PubPeer Demand Letter, unlike the other "Anversa Inquiry/Investigation," purported to notify Dr. Houser that after an unspecified "preliminary assessment," Temple "has initiated an inquiry" under Temple's Creative Work Policy and federal regulations.

191. Notably, Temple did not appear to conduct any "preliminary assessment" investigation as it did with the Anversa Inquiry/Investigation. Further, Masucci made no reference in the PubPeer Demand Letter to any apparent conflict of interest that she might have had with Dr. Houser. Thus, whatever conflict of interest she referenced relating to the Anversa Inquiry/Investigation, Masucci seemed to have resolved it with this second Inquiry.

192. Further, Temple did not identify any outside counsel that assisted it with the unspecified "preliminary assessment."

193. By email dated January 13, 2021, Masucci notified Dr. Houser that since he did not state that he wanted to meet again with Masucci's "team" respecting the Anversa matter, "I

want to let you know that we are moving forward without your further input related to the preliminary assessment."

194. The Anversa Inquiry/Investigation has been ongoing since September 2019 and continues to this day.

195. Over that time, Temple's purported "preliminary assessment" has involved the interrogation of Dr. Houser for over 5 hours, demanding voluminous documents and related information – without his knowledge or consent – from his lab colleagues, collecting certain documents and information, demanding additional interrogations with Temple's interrogation team, Wilmer Hale, and ignoring Dr. Houser's every request for clarity, information and the basis and foundation for Temple's actions.

196. Contrary to Temple's express Creative Work Policy, this "Inquiry" or "Investigation" or whatever it the process is called by Temple, the Anversa Inquiry/Investigation has not been completed within 60 or 90 days or 120 or 180 days from its inception. *See* Creative Work Policy at Policy, sec. J.

197. In contravention of Temple's own Creative Work Policy, at no time up to and including the filing of this Complaint was Dr. Houser ever formally notified by Temple or its Integrity Officer (1) in writing, that an Inquiry Committee was to be convened to consider an allegation of misconduct, (2) informed of the nature of the allegation, (3) provided a copy of the Creative Work Policy, or (4) notified that he will have the opportunity to be interviewed by and present evidence to the Inquiry Committee. *See* Creative Work Policy at Procedures, Art. I.B.1.

198. The frivolous Anversa Inquiry/Investigation has badly damaged Dr. Houser's reputation and continues to badly damage his reputation and position of authority at the School of Medicine and in the greater academic community.

199. As a direct result of Temple's illegal actions, Dr. Houser has not taken on any new leadership roles in any professional organizations because the ongoing investigation would have a negative impact on the organizations he would be representing.

200. Temple's conduct, moreover, comes at a time when Dr. Houser is planning for his career after Temple.

201. Temple's unfounded and undefined Anversa Inquiry/Investigation continues to negatively impact all plans Dr. Houser has for his career after leaving Temple.

202. Contrary to Masucci's statements, there is no authority under the Creative Work Policy for a preliminary assessment to include full-scale investigations, respondent interviews, demands for documents, and outsourcing the investigatory process.

203. By letter dated January 22, 2021, Dr. Houser, through his counsel, responded to each purported allegation in the PubPeer Demand Letter, denouncing, among other things, Temple's continued failure to identify the specific basis on which Temple was pursuing documents and information against Dr. Houser.

204. Dr. Houser specifically demanded to know whether Temple was acting pursuant to an Inquiry or Investigation under the Creative Work Policy.

205. Dr. Houser also demanded to know whether any allegation existed outside of the PubPeer Claims.

206. Further, Dr. Houser specifically detailed his role, such as it was, in each of the 9 papers identified by Temple, noting that for 5 of the papers, Dr. Houser merely ***edited*** the text of certain sections for a non-native English speaking author. Dr. Houser provided no data from his laboratory, analyzed no data for the paper, and did not design or oversee any of the experiments described in them.

207. Respecting one paper – the one that PubPeer shows was corrected – Dr. Houser explained that the figure shown in the paper had a duplication due to a clerical error. The error

was corrected (as confirmed on PubPeer), had no impact on the methodology or conclusion of the paper, and was otherwise a non-event.

208. One of the papers, in fact, was completely appropriate and the PubPeer "allegation" lacked any "strong evidence" of any problem, dishonesty or mistakes.

209. Another paper, relating to mouse RNA, was wrongly trolled by a commenter on PubPeer as involving human RNA.

210. Dr. Houser also repudiated the "allegation" relating to a final paper identified by Temple, noting that he had nothing to do with any of the experiments in the study.

211. In response, Temple merely acknowledged receipt of Dr. Houser's refutations and stated that it "will respond in due course."

212. To this day, the PubPeer Claims and Anversa Inquiries/Investigations continue without providing any substantive basis for either or any authority under Temple's Creative Work Policy for Temple's actions against Dr. Houser.

213. In the meantime, Temple has not responded in any substantive manner respecting Dr. Feldman's use of the Stolen IP and his defamatory statements about Houser.

214. For the reasons that follow, however, Temple and Dr. Feldman are in business together, standing to potentially reap millions of dollars in revenue, while using Dr. Houser's Stolen IP.

Temple and Feldman Improperly Rely Upon Dr. Houser's Research in Patent Applications and Fail to Name Him as a Co-Inventor

215. In late 2019, Dr. Houser learned that Dr. Feldman's company, Renovacor, Inc. ("Renovacor"), which is pursuing treatments for heart failure utilizing BAG3, had received significant private equity financing. Knowing the importance of patents to obtaining such financing, Dr. Houser became concerned for the first time that the Stolen IP might have been used in patent filings, even though he had made Temple aware that the Stolen IP belonged to him and not Dr. Feldman.

216. Accordingly, still believing that the University would handle this matter appropriately, Dr. Houser asked Masucci if his Stolen IP was being used by Dr. Feldman in any patent filings. Dr. Houser was told by Masucci that he needed to wait until the conclusion of the investigation before he could get an answer to that question.

217. Upon information and belief, Masucci, on behalf of Temple, attempted to deliberately mislead Dr. Houser into believing he could obtain no additional information regarding the potential use of the Stolen IP in patents at that time and, further, that Temple would address this issue appropriately at that time if needed.

218. Over time in 2020, Dr. Houser became skeptical of the University's good faith in handling this matter, and thus began his own an investigation into whether the Stolen IP was used in any patent filings.

219. Thus, in November 2020 Dr. Houser learned that not only did Dr. Feldman take the Stolen IP and pass it off as his own in a publication, but also Temple and Dr. Feldman had indeed compounded the injury to Dr. Houser by including Dr. Houser's Stolen IP in several patent applications in the United States and other countries.

220. The patent applications filed by Temple appear to be for a groundbreaking innovation (if successful) in the treatment of heart failure. Upon information and belief, Renovacor is further exploiting the Stolen IP in its development of its treatment of heart failure, driving significant private equity investment into Renovacor in 2019, as well as its merger earlier this year.

221. These applications name Dr. Feldman as the lead inventor and omit Dr. Houser altogether as a co-inventor.

222. Specifically and unbeknownst to Dr. Houser until 2020, Dr. Feldman included Dr. Houser's Stolen IP in the patent application for methods of treating patients with heart failure

and a reduced level of BAG3, U.S. Patent Application No. 15/115,807, titled "BAG3 As a Target for Therapy of Hearth Failure," (the "'807 Application"), attached hereto as Exhibit "D."

223. More specifically, Dr. Houser's Stolen IP appears in Figures 7A-7E in the published patent application and is specifically discussed in paragraph [0254].

224. Dr. Houser's Pig Data appears in Figures 7A-7D, which includes functional measurements of the heart (including ejection fraction) of a pig where myocardial infarction (heart attack) was induced compared to a control (where myocardial infarction was not induced). These figures were the data prepared by Sharp at Feldman's request.

225. Figures 7E and 7F include the protein measurements Feldman conducted on Dr. Houser's Pig Model Tissue Samples that show reduced levels of BAG3 in the pig where myocardial infarction was induced compared to a control.

226. Notably, the data included in the '807 Application, is identical to that used by Dr. Feldman in the Feldman Paper and includes Dr. Houser's Pig Data as well as the BAG3 protein level measurements Feldman derived from Dr. Houser's Pig Model Tissue Samples.

227. Not only was Dr. Houser's Stolen IP used and published again without his knowledge and permission, he was also not named – as he should have been – as a co-inventor on the '807 Application.

228. Instead, the '807 Application only lists Dr. Feldman, Douglas Tilley, Weizhong Zhu, Kamel Khalili, and Walter Koch as the named inventors, with Temple University as applicant and assignee.

229. The '807 Application includes broad claims directed to methods of treating patients suffering from heart failure with reduced ejection fraction that also express a decreased level of BAG3 protein.

230. As described above, Dr. Houser's Stolen IP is the animal model that established the clinical legitimacy and significance of BAG3 as a potential therapeutic target for patients suffering from ischemic heart failure.

231. A decreased level of expression of BAG3 is a critical clinical element of the pending claims.

232. Without Dr. Houser's Stolen IP, Dr. Feldman would not have the necessary support for the '807 Application's broad claims given that only a small subset of the population of relevant patients suffer from heart failure caused by Dr. Feldman's discovered genetic mutation, and the mouse model included in the specification does not (and cannot) mimic what occurs when a patient suffers a heart attack.

233. Dr. Houser was unaware of the filing of the '807 Application, including the use of his Stolen IP, at the time it was filed and never gave permission for the use of the Stolen IP or any of his research and data to be used in the '807 Application.

234. Dr. Houser only became aware that his research was included in the '807 Application on November 17, 2020, when a colleague assisted him in searching for patent filings.

235. Dr. Houser's Stolen IP is an essential piece of the claimed invention for at least two reasons: (1) it is a large animal model of heart failure with reduced ejection fraction that closely mimics what happens in human patients that suffer a heart attack, the most common form of ischemic heart failure, and (2) it confirms that in such situations, the BAG3 level is decreased.

236. Upon information and belief, the Stolen IP and information related to it was used and relied upon by Temple in prosecuting the '807 Application and associated patent applications.

237. Without Dr. Houser's Stolen IP, the pending claims towards methods of treatment for patients with heart failure with reduced ejection fraction and a decreased level of BAG3, lack sufficient support to be valid.

238. Without Dr. Houser as a named inventor on the pending application, the claims are invalid as patent examiners are instructed to reject applications with incorrect inventorship under MPEP § 2157.

239. Further, on information and belief, Dr. Houser was intentionally omitted as an inventor on the pending application with intent to deceive the US Patent Office, thus rendering any patent that issues unenforceable due to inequitable conduct.

240. Despite the fact that Dr. Houser's Stolen IP is essential to the validity of the pending claims (which undoubtedly was the reason for its misappropriation and inclusion in the application), inventorship of the '807 Application has never been corrected.

241. A notice of allowance for the '807 Application, with incorrect inventorship, was issued on September 8, 2020.

242. The '807 Application, however, remains unissued and is continuing to be examined by the USPTO. A non-final rejection was issued by the USPTO on April 15, 2021 (the "Office Action").

243. In the Office Action, all of the pending claims in the '807 Application "are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-6, 8, 25, 26 of copending Application No. 15/929,784 (reference application)."

244. Application No. 15/929,784 was filed by Temple University on May 21, 2020, titled "BAG3 As a Target for Therapy of Heart Failure" was published on January 21, 2021 (the "'784 Application").

245. The '784 Application lists Feldman, Tilley, Zhu, Khalili, and Koch as inventors, and is attached hereto as Exhibit E.

246. The '784 Application also uses and relies on Dr. Houser's Stolen IP. Ex. E, Figs. 7A-7F, ¶¶ [0021], [0268].

247. In addition to using Dr. Houser's Stolen IP as part of the invention forming the basis of the '807 and '784 Applications, upon information and belief, Temple and Feldman also filed companion patent applications on the invention claimed in the '807 Application in the European Patent Office (EP 3099333), Japan (Application No. 20165493334), and Canada (Application No. 2975258).

Dr. Houser Has Been Injured Economically by not Being Named a Co-Inventor

248. As part of Temple's policies, academic inventors of certain technologies or applications agree that Temple owns the intellectual property as a "work for hire" but that the individual inventors can benefit financially if the invention is monetized. This monetization usually occurs when a company licenses (typically exclusively) the invention in question from Temple.

249. Under Temple's Inventions Policy, fifty percent 50% of the net income from each invention is distributed among the named inventors. Typically, the named inventors come to agreement among themselves as to the distribution of the inventors' share of the net income.

250. By omitting Dr. Houser as a named inventor on the '807 and '784 Applications, Dr. Houser is excluded from sharing in any proceeds that result from the monetization of the claimed invention.

251. Due to the importance of Dr. Houser's contribution to the invention claimed in the '807 and '784 Applications, upon information and belief, he likely would have received a significant portion of the inventors' share of the net income from the invention.

252. Upon information and belief, Renovacor has licensed the invention claimed in the '807 Application from Temple. Upon information and belief, Temple has received or will receive licensing royalties, including perhaps at least a portion of the investment that Renovacor

received from Broadview Ventures and/or is entitled to receive a percentage of any future sales of Renovacor products that commercialize the invention claimed in the '807 and '784 Applications.

253. Upon information and belief, without the Stolen IP which made the invention possible and enhanced its commercial viability, Renovacor would not have received investment (or any promised investment or capital) from Broadview Ventures.

254. Given the nature of the invention, upon information and belief Renovacor, Temple, Feldman and the other inventors have and/or will derive substantial income from the invention claimed in the '807 and '784 Applications.

255. As an omitted co-inventor, Dr. Houser has thus been deprived of the opportunity to earn his rightful share of this revenue.

Temple's Inquiries/Investigations Are Mere Pretext to Deprive, Threaten and Intimidate Dr. Houser into Allowing Temple to Take the Stolen IP for Its and Dr. Feldman's Benefit

256. Upon information and belief, Temple's Inquiries/Investigations constitute a mere pretext to malign, harass, threaten and intimidate Dr. Houser into dropping pursuit of his rights under Temple's applicable policies and procedures to clear his name and into dropping his pursuit of his rights to the Stolen IP, Dr. Feldman's defamatory statements about Dr. Houser, and his rights to financial remuneration concerning Renovacor and the related patent filings.

257. Upon information and belief, Temple's Inquiries/Investigations into Dr. Houser are being pursued in bad faith and are not being conducted in accordance with the applicable Temple policies and procedures.

258. Upon information and belief, Temple's refusals to pursue any Inquiries/Investigations into any of the complaints made by Dr. Houser against Dr. Feldman and the direct statement that Masucci wanted to conclude the inquiries/investigations into Dr. Houser before looking into any of his allegations respecting Dr. Feldman constitute harassment and

intimidation and an improper method of forcing Dr. Houser to accept Dr. Feldman's misconduct and the economic relationship between Renovacor and Temple.

259. Defendants' conduct as described herein is illegal and unlawful, for which they are liable to Dr. Houser.

COUNT I – BREACH OF CONTRACT

260. Dr. Houser incorporates herein by reference each of the foregoing allegations.

261. Temple and Dr. Houser are parties to the 2012 Agreement and 2017 Agreement, which, among other things, incorporate the Creative Work Policy and Inventions Policy.

262. The Creative Work Policy and Inventions Policy are binding and enforceable agreements between Temple and Dr. Houser.

263. As stated above, Temple, through its improper (and/or unauthorized or baseless) Inquiries/Investigations and failures to act upon Dr. Houser's complaints about the Stolen IP and defamatory statements disseminated by Dr. Feldman, failed to honor the terms of the various agreements between Temple and Dr. Houser.

264. Dr. Houser has suffered and will continue to suffer damages as a result of Temple's failures.

265. As stated above, Temple's failures constitute breaches of the applicable agreements, for which it is liable to Dr. Houser.

266. Dr. Houser, to the extent applicable, satisfied all conditions precedent under the agreements entitling him to pursue these claims against Temple.

WHEREFORE, Dr. Houser requests that the Court enter judgment in his favor and against Temple in an amount in excess of $150,000 and grant him such other relief deemed appropriate under the circumstances.

COUNT II – MISAPPROPRIATION OF TRADE SECRETS (FEDERAL DEFEND TRADE SECRETS ACT, 18 U.S.C. § 1831, ET SEQ.)

267. Dr. Houser incorporates herein by reference each of the foregoing allegations.

268. Dr. Houser has equitable title to and/or a license in the Stolen IP and, as such, has standing to pursue this claim. In particular, although Dr. Houser was obligated to transfer his ownership of the Stolen IP to Temple, under Temple's policies, he was able to maintain the Stolen IP in confidence and control the use or non-use of the Stolen IP within his academic work. Dr Houser maintains both an economic interest in the Stolen IP as an inventor, and also an equitable interest in the Stolen IP as an academic, under doctrines of academic integrity and integrity of scholarship, which are the fundamental underpinnings of all academic research institutions.

269. As described above, Defendants were fully familiar with Dr. Houser's Stolen IP.

270. As described above, Dr. Feldman, under fraudulent circumstances, acquired Dr. Houser's Stolen IP for use in the Feldman Paper and later, both Dr. Feldman and Temple used the Stolen IP in the various patent filings. Upon information and belief, Dr. Feldman is also utilizing and relying upon the Stolen IP in his work at Renovacor and in obtaining private equity financing for Renovacor. Upon information and belief, the Stolen IP was also material to the recent merger between Renovacor and Chardan Healthcare Acquisition 2 Corp.

271. Dr. Houser never agreed to allow any Defendant to acquire, disclose, or use the Stolen IP for any purpose.

272. Dr. Houser had taken reasonable efforts to maintain the secrecy of the Stolen IP, including, but not limited to, keeping it in secured locations sharing the information on a limited, need-to-know basis and not disclosing it to any third-parties without appropriate precautions.

273. Dr. Houser's Stolen IP obviously have significant economic and commercial value, representing an investment of many years and millions of dollars, and are not generally known or readily ascertainable by others.

274. Dr. Feldman and later Temple relied upon and used Dr. Houser's Stolen IP without Dr. Houser's permission when, for Dr. Feldman's purpose, publishing the Feldman Paper and when drafting the '807 and '784 Applications for the sole benefit of Defendants.

275. Upon information and belief, Defendants are continuing to rely upon and use the Stolen IP in further development and prosecution of the '807 and '784 Applications.

276. Dr. Houser did not discover the use of the Stolen IP in the '807 Application until November 2020.

277. Dr. Houser was not aware of the use of the Stolen IP in the '784 Application until April 2021.

278. Dr. Houser had no reason to know of the use of the Stolen IP in any patent applications prior to November 2020.

279. Further and in the alternative, prior to November 2020 and relied upon in good faith Temple's representations regarding the handling and use of the Stolen IP. In particular, Dr. Houser, based on statements of Masucci, believed that the matter would be handled appropriately in accordance with Temple's policies including, *inter alia*, regarding the appropriate compensation of co-inventors. Dr. Houser thus believed that the matter was fully resolved and no further use would be made of the Stolen IP. Upon information and belief, Defendants' misappropriation of Dr. Houser's Stolen IP saved them considerable time and monetary investment in the development and prosecution of the '807 and '784 Applications.

280. Defendants' improper activities, described above, were a knowing, willful, malicious and deliberate disregard of Dr. Houser's rights and interests. Such conduct warrants an award of exemplary damages in an amount up to two times the damages awarded and an award of reasonable attorneys' fees to Dr. Houser.

281. Dr. Houser has suffered and will continue to suffer material damage as a result of this continued misuse of the Stolen IP.

282. Defendants are therefore liable to Dr. Houser for such damages.

WHEREFORE, Dr. Houser requests that the Court enter judgment in his favor and against Defendants in an amount in excess of $150,000, grant Dr. Houser an award of reasonable attorneys' fees and exemplary damages as provided for under 18 U.S.C. § 1831, et seq., that the Court enter an injunction against Defendants, directing and ordering the following:

(a) Direct Temple to immediately correct the patent applications (and the foreign counterparts) to add Dr. Houser as a co-inventor;

(b) Direct Dr. Feldman to immediately issue a retraction and correction of the Feldman Paper to recognize Dr. Houser as author/creator/inventor of the Stolen IP; and

(c) Direct Dr. Feldman and Temple to immediately amend all agreements relating to Renovacor and any aspect of the Stolen IP to add Dr. Houser as a party or recipient of monetary or other compensation for use and inclusion of the Stolen IP. To the extent there exists any dispute about the percentage interest attributable to Dr. Houser, such dispute shall be presented to the Court if the parties cannot successfully negotiate it amongst themselves;

and grant him such other relief deemed appropriate under the circumstances.

COUNT III – MISAPPROPRIATION OF TRADE SECRETS (PENNSYVLANIA UNIFORM TRADE SECRETS ACT, 12 PA.C.S. § 5301, ET SEQ.)

283. Dr. Houser incorporates herein by reference each of the foregoing allegations.

284. Dr. Houser enjoyed a valid property interest in the Stolen IP, including but not limited to lawful possession of the Stolen IP and the right to enjoy the value of the secrecy of the Stolen IP and, as such, has standing to pursue this claim. In particular, although Dr. Houser was obligated to transfer his ownership of the Stolen IP to Temple, under Temple's policies, he was able to maintain the Stolen IP in confidence and control the use or non-use of the Stolen IP within his academic work. Dr Houser maintains both a pecuniary interest in the Stolen IP as an inventor, and also an equitable interest in the Stolen IP as an academic, under doctrines of academic integrity and integrity of scholarship, which are the fundamental underpinnings of all academic research institutions.

285. As described above, Defendants were fully familiar with Dr. Houser's Stolen IP.

286. As described above, Dr. Feldman, under fraudulent circumstances, acquired Dr. Houser's Stolen IP for use in the Feldman Paper and later in the various patent filings.

287. Dr. Houser never agreed to allow any Defendant to disclose or use the Stolen IP for any purpose.

288. Dr. Houser had taken reasonable efforts to maintain the secrecy of the Stolen IP, including, but not limited to, sharing the information on a limited, need-to-know basis and not disclosing it to any third-parties without appropriate precautions.

289. Dr. Houser's Stolen IP obviously have significant economic and commercial value, representing an investment of many years and millions of dollars, and are not generally known or readily ascertainable by others.

290. Dr. Feldman and later Temple relied upon and used Dr. Houser's Stolen IP without Dr. Houser's permission when, among other things, for Dr. Feldman's purpose, publishing the Feldman Paper and when drafting the '807 and '784 Applications for the sole benefit of Defendants.

291. Upon information and belief, Defendants are continuing to rely upon and use the Stolen IP in further development and prosecution of the '807 and '784 Applications.

292. Upon information and belief, Defendants' misappropriation of Dr. Houser's Stolen IP saved them considerable time and monetary investment in the development and prosecution of the '807 and '784 Applications.

293. Defendants' improper activities, described above, were a knowing, willful, malicious and deliberate disregard of Dr. Houser's rights and interests. Such conduct warrants an award of exemplary damages in an amount up to two times the damages awarded and an award of reasonable attorneys' fees to Dr. Houser.

294. Dr. Houser has suffered and will continue to suffer material damage as a result of this misuse of the Stolen IP.

295. Defendants are therefore liable to Dr. Houser for such damages.

WHEREFORE, Dr. Houser requests that the Court enter judgment in his favor and against Defendants in an amount in excess of $150,000, grant Dr. Houser an award of reasonable attorneys' fees and exemplary damages as provided for under 12 PA.C.S. § 5301, et seq., that the Court enter an injunction against Defendants, directing and ordering the following:

(a) Direct Temple to immediately correct the patent application (and the foreign counterparts) to add Dr. Houser as a co-inventor;

(b) Direct Dr. Feldman to immediately issue a retraction and correction of the Feldman Paper to recognize Dr. Houser as author/creator/inventor of the Stolen IP; and

(c) Direct Dr. Feldman and Temple to immediately amend all agreements relating to Renovacor and any aspect of the Stolen IP to add Dr. Houser as a party or recipient of monetary or other compensation for use and inclusion of the Stolen IP. To the extent there exists any dispute about the percentage interest attributable to Dr. Houser, such dispute shall be presented to the Court if the parties cannot successfully negotiate it amongst themselves; and

(d) Grant him such other relief deemed appropriate under the circumstances.

(a)

COUNT IV – SPECIFIC INJUNCTIVE RELIEF

296. Dr. Houser incorporates herein by reference each of the foregoing allegations.

297. While Dr. Houser believes he may have certain legal remedies at law, as stated herein above and after, Dr. Houser avers that he also is entitled to the imposition of the following injunctive relief for which he has no adequate remedy of law due to Defendants' acts or omissions as averred herein.

298. Dr. Houser is and has been an employee of Temple and, for a time, a subordinate of Dr. Feldman.

299. As an employee at Temple, Dr. Houser has various rights, agreed to expressly by Temple, to the recognition and protection of intellectual property created by Dr. Houser through his efforts at Temple, the prompt and orderly inquiry or investigation into issues of misconduct,

plagiarism or other academic dishonesty, to the fair treatment of his rights and interests, and to certain monetary arrangements for any works or intellectual property created by or through his efforts.

300. Dr. Houser has made demands to Defendants to, on the one hand, account for the Stolen IP and, on the other hand, to cease the Anversa and PubPeer Demand investigations and to commence an investigation into Dr. Feldman's defamatory statements and other misconduct as described above.

301. In response to these demands, Defendants have unjustifiably refused to return the Stolen IP, recognize Dr. Houser's interests in the Stolen IP, and as to Temple, it has unjustifiably refused to cease the baseless inquiries/investigations against Dr. Houser, provide Dr. Houser with the appropriate information and access under Temple's own policies, initiate any inquiry/investigation into Dr. Houser's complaints about the Stolen IP, his rights thereunder, or Dr. Feldman's defamatory statements about Dr. Houser.

302. Further, Defendants' actions violate the aforementioned agreements and related statutory and common laws respecting Dr. Houser's various rights, as described above.

303. Dr. Houser will suffer immediate and irreparable harm in the deprivation of his rights, as described above, if he is not granted the injunctive relief requested herein.

304. Dr. Houser does not have an adequate remedy at law for the injuries he has and will continue to suffer as a result of Defendants' continued violation of his rights as stated above.

305. Greater injury will result from refusing the relief requested herein than from granting it, as Dr. Houser will continue to be deprived of important rights if the relief is denied, but Defendants will be able to function normally if the relief is granted.

306. The injunctive relief will restore Dr. Houser to the position he was in prior to Defendants' wrongful conduct.

307. Dr. Houser is likely to prevail on the merits of his claims.

308. The relief requested herein is reasonably suited to abate the offending activity, as it directly requires Defendants to cease from continuing the offending activities, and prevents Defendants from causing harm to Dr. Houser and his rights.

309. The injunction will not adversely affect the public interest.

WHEREFORE, Dr. Houser respectfully requests that the Court enter an injunction against Defendants, directing and ordering the following:

(a) Direct Temple to immediately correct the patent application (and the foreign counterparts) to add Dr. Houser as a co-inventor;

(b) Direct Dr. Feldman to immediately issue a retraction and correction of the Feldman Paper to recognize Dr. Houser as author/creator/inventor of the Stolen IP;

(c) Direct Dr. Feldman and Temple to immediately amend all agreements relating to Renovacor and any aspect of the Stolen IP to add Dr. Houser as a party or recipient of monetary or other compensation for use and inclusion of the Stolen IP. To the extent there exists any dispute about the percentage interest attributable to Dr. Houser, such dispute shall be presented to the Court if the parties cannot successfully negotiate it amongst themselves;

(d) Direct Dr. Feldman to immediately cease and forever desist in defaming or libeling or communicating anything derogatory, defamatory, or intending to malign or criticize Dr. Houser's character or reputation. To the extent that Dr. Feldman has printed any such statements, he shall immediately delete, retract and destroy any such writings and confirm in a Declaration or Affidavit to this Court all the places such statements were published and precisely how such communications were retracted and destroyed or deleted;

(e) Direct Temple to immediately cease all "preliminary assessments," "inquiries," or "investigations" into or against Dr. Houser and to the extent any such events are in progress or contemplated, Temple shall immediately provide Dr. Houser all claims against or concerning him, in detail and with specificity as to the nature of the claims, the topics or subjects at issue and Temple's efforts to the present to assess, inquire or investigate such matters; and

(f) Such further relief as this Court may deem just and proper under the circumstances shall be afforded.

COUNT V – CONSTRUCTIVE TRUST

310. Dr. Houser incorporates herein by reference each of the foregoing allegations.

311. To the extent that the injunctive relief sought by Dr. Houser is denied, Dr. Houser is entitled to the imposition of a constructive trust against Defendants for the preservation of the Stolen IP and any monies received as a result of any efforts which used or incorporated the Stolen IP until this matter has ended.

312. Dr. Houser has a legal, tangible and personal interest in the Stolen IP and any monies derived from their use or incorporation into any other property, concept, business or idea, and he is entitled to compensation for his interest therein.

313. Defendants have an adverse interest to Dr. Houser with regard to such funds.

314. An actual controversy exists between the parties concerning their respective interests in the Stolen IP.

315. By virtue of Defendants' acts and omissions, they are in possession and total control of any monies received relating to the use or incorporation of the Stolen IP and the Stolen IP itself on a daily basis.

316. Defendants will be in possession and control of all such items in the future and during the pendency of this lawsuit.

317. The retention of such funds and property constitutes an unjust enrichment to Defendants.

318. Dr. Houser is entitled to the imposition of a constructive trust over these assets to protect their interest during the pendency of this litigation.

319. Dr. Houser is entitled to the imposition of a constructive trust over the assets described above to protect his interests.

320. If a constructive trust is not imposed by this Court, Defendants may distribute and dissipate such funds or property to the continued detriment of Dr. Houser.

WHEREFORE, Dr. Houser respectfully requests that this Court:

(a) Order the imposition of a constructive trust as to all the assets of Defendants that relate, in any way, to the Stolen IP during the pendency of this litigation;

(b) Enter judgment declaring that Dr. Houser has a protectable and enforceable interest in such property;

(c) Enjoin Defendants from using or distributing any funds derived from any part or portion of the Stolen IP or the '807 and '784 Applications during the pendency of this litigation without further order and permission of this Court;

(d) Award Dr. Houser his costs of litigation, incurred as a result of this action; and

(e) Grant such other further relief as this Court deems appropriate.

COUNT VI – ACCOUNTING

321. Dr. Houser incorporates herein by reference each of the foregoing allegations.

322. To the extent that the injunctive relief sought by Dr. Houser is denied, Dr. Houser is entitled to an accounting of the financial records of Temple to determine his interest in and any pecuniary remuneration to which he might be entitled as a result of the Stolen IP.

323. Dr. Houser is an owner or inventor of the Stolen IP.

324. As described above, Defendants, through Dr. Feldman, misappropriated the Stolen IP, under fraudulent circumstances, and have denied Dr. Houser's rights, interests, and contributions.

325. Dr. Houser has made repeated demands to Temple asking for an inquiry or investigation into the Stolen IP to no avail.

326. Under the applicable Temple policies, Dr. Houser has a right to ownership, authorship or inventor's credits for the Stolen IP as well as rights for certain monetary remuneration.

327. Temple's failures and refusals to investigate the Stolen IP claims, recognize Dr. Houser's rights and arrange for his financial interest in such rights constitute a material breach of the terms of the agreements between Temple and Dr. Houser.

WHEREFORE, Dr. Houser respectfully requests that this Court enter an order allowing Dr. Houser full, complete and unfettered access to the financial records of Temple as they pertain to Renovacor, Dr. Feldman and the Stolen IP to conduct an accounting of such records and his interests in accordance with his contractual rights; and grant such other further relief as this Court deems appropriate.

COUNT VII - UNJUST ENRICHMENT

328. Dr. Houser incorporates herein by reference each of the foregoing allegations.

329. If and to the extent that the express agreements between Temple and Dr. Houser (and agreements to which Dr. Feldman might also be a party) are either found not to be valid and enforceable or applicable to the unlawful taking of the Stolen IP, then Dr. Houser is nevertheless entitled to recover damages from Defendants under the theory of unjust enrichment.

330. Also, in addition – and specifically in the alternative to Counts II and III regarding misappropriation of trade secrets -- Dr. Houser is nevertheless entitled to recover damages from Defendants under the theory of unjust enrichment.

331. As described above, Dr. Houser, through the Stolen IP, conferred benefits on Defendants.

332. As described above, Defendants appreciated, acknowledged and retained the benefits conferred on them by the Stolen IP.

333. The acceptance and retention by Defendants of all of the benefits conferred on them by Dr. Houser, under the circumstances, would be inequitable in the absence of full payment to Dr. Houser for the value of all of the benefits conferred.

WHEREFORE, Dr. Houser requests that the Court enter judgment in his favor and against Defendants in an amount in excess of $150,000 and grant him such other relief deemed appropriate under the circumstances.

COUNT IX - CONVERSION

334. Dr. Houser incorporates herein by reference each of the foregoing allegations.

335. Pursuant to the agreements between Temple and Dr. Houser, Temple and Dr. Feldman were required to recognize and provide for Dr. Houser's financial and proprietary interests in the Stolen IP, and to the extent money has been paid, Temple and Dr. Feldman were required to pay Dr. Houser.

336. Further, as described above, Dr. Houser had an express, recognized ownership, creator, or inventor's interest in the Stolen IP; Dr. Feldman had none.

337. As described above, Dr. Feldman and Temple have exercised complete ownership and control over the Stolen IP, which was secured under fraudulent circumstances, and which was not authorized or agreed to by Dr. Houser.

338. Dr. Houser's rights in the Stolen IP have been violated, and Defendants' acts or omissions, as described above, constitute conversion of monies and property rights rightfully due and owing to Dr. Houser, for which they are liable to Dr. Houser.

339. Defendants' conduct was outrageous, wanton and willful.

340. Punitive damages are warranted to punish Defendants and deter them from engaging in such conduct in the future.

WHEREFORE, Dr. Houser requests that the Court enter judgment in his favor and against Defendants in an amount in excess of $150,000 in compensatory damages, award him punitive damages against Defendants, that the Court enter an injunction against Defendants, directing and ordering the following:

(a) Direct Temple to immediately correct the patent application (and the foreign counterparts) to add Dr. Houser as a co-inventor;

(b) Direct Dr. Feldman to immediately issue a retraction and correction of the Feldman Paper to recognize Dr. Houser as author/creator/inventor of the Stolen IP; and

(c) Direct Dr. Feldman and Temple to immediately amend all agreements relating to Renovacor and any aspect of the Stolen IP to add Dr. Houser as a party or recipient of monetary or other compensation for use and inclusion of the Stolen IP. To the extent there exists any dispute about the percentage interest attributable to Dr. Houser, such dispute shall be presented to the Court if the parties cannot successfully negotiate it amongst themselves; and

(d) Grant him such other relief deemed appropriate under the circumstances.

COUNT X - CIVIL CONSPIRACY

341. Dr. Houser incorporates herein by reference each of the foregoing allegations.

342. As described above, Defendants acted in concert with one another to deprive Dr. Houser of his ownership and monetary rights to the Stolen IP, so that Defendants could use the Stolen IP to earn significant monies (and thereby increase their own respective shares and prestige)

343. For Dr. Feldman's part, he defamed Dr. Houser, secured the Stolen IP under fraudulent circumstances, and otherwise passed off the work of others as his own for his own personal benefit.

344. For Temple's part, it conducted various, baseless investigations – designated from a "preliminary assessment" to an "inquiry" – all in an effort to discredit and threaten Dr. Houser into inaction.

345. Further, Temple threatened Dr. Houser that his complaints about Dr. Feldman would have to wait until Temple finished its investigations into Dr. Houser, thereby creating the impression that Dr. Houser's cooperation in unfounded investigations and keeping his mouth shut over Dr. Feldman's misconduct would be in his best interest as opposed to getting at the truth of the matters.

346. As described above, it is clear that Temple and Dr. Feldman, through the alleged "apology" over the Stolen IP and the filing and prosecution of the '807 and '784 Applications

and the contract with Renovacor, have agreed and committed another overt act to purse the common plan or design of freezing out Dr. Houser and enriching themselves.

347. As described above, Defendants' acts and omissions were committed with malice and an intent to injure or harm Dr. Houser or his interests.

348. As described above, Defendants' acts and omissions were committed without justification.

349. Defendants' acts or omissions, as described above, were outrageous, wanton and willful.

350. Punitive damages are warranted to punish Defendants and deter them from engaging in such conduct in the future.

WHEREFORE, Dr. Houser requests that the Court enter judgment in his favor and against Defendants in an amount in excess of $150,000 in compensatory damages, award him punitive damages against Defendants, that the Court enter an injunction against Defendants, directing and ordering the following:

(a) Direct Temple to immediately correct the patent application (and the foreign counterparts) to add Dr. Houser as a co-inventor;

(b) Direct Dr. Feldman to immediately issue a retraction and correction of the Feldman Paper to recognize Dr. Houser as author/creator/inventor of the Stolen IP; and

(c) Direct Dr. Feldman and Temple to immediately amend all agreements relating to Renovacor and any aspect of the Stolen IP to add Dr. Houser as a party or recipient of monetary or other compensation for use and inclusion of the Stolen IP. To the extent there exists any dispute about the percentage interest attributable to Dr. Houser, such dispute shall be presented to the Court if the parties cannot successfully negotiate it amongst themselves; and

(d) Grant him such other relief deemed appropriate under the circumstances.

COUNT XI – DEFAMATION (AGAINST DR. FELDMAN ONLY)

351. Dr. Houser incorporates herein by reference each of the foregoing allegations.

352. As described above, Dr. Feldman made communications to various colleagues of Dr. Houser, including, but not limited, Patrick O'Connor, and others, which communications were defamatory in character.

353. The communications made by Dr. Feldman were defamatory in that they tended to harm Dr. Houser's reputation so as to lower him in the estimation of the community or to deter third persons from associating or dealing with him.

354. In an academic society of the like Dr. Houser has spent his entire professional career working and thriving in, these defamatory statements were especially harmful and damaging.

355. As described above, Dr. Houser (and indeed Temple itself) relied on Dr. Houser's research and reputation to secure grants, projects, and other opportunities designed to increase the notoriety of Temple and the School of Medicine or provide for funds or access to prestigious appointments, programs or research.

356. As described above, Dr. Feldman made the communications to apply to Dr. Houser and to imbue the understanding in the recipient that Dr. Feldman either committed an act or omission questioning his character, ethics or credibility and otherwise maligning Dr. Houser's professional and personal reputations.

357. Dr. Feldman's communications were published to third-parties and he had no privilege or justification in making such communications.

358. Dr. Feldman's conduct was outrageous, wanton and willful.

359. Punitive damages are warranted to punish Dr. Feldman and deter him from engaging in such conduct in the future.

WHEREFORE, Dr. Houser requests that the Court enter judgment in his favor and against Dr. Feldman in an amount in excess of $150,000 in compensatory damages, award him punitive damages against Dr. Feldman and grant him such other relief deemed appropriate under the circumstances.

WISLER PEARLSTINE, LLP

Date: May 31, 2021

By: /s/ David M. Burkholder
David M. Burkholder, Esquire
460 Norristown Road, Suite 110
Blue Bell, PA 19422
dburkholder@wispearl.com
Attorneys for Plaintiff

EXHIBIT "A"

School of Medicine
TEMPLE UNIVERSITY

Senior Executive VP, Health Sciences
President and CEO
Temple University Health System

3500 N. Broad Street
Medical Education Research Building
Suite 1141
Philadelphia, PA 19140

phone 215-707-8773
fax 215-707-8431
e-mail larry.kaiser@tuhs.temple.edu
web www.temple.edu/medicine

March 20, 2012

Steven R. Houser, Ph.D.
1168 Kenyon Drive
Fort Washington, PA 19034-1628

Dear Dr. Houser:

I am pleased to confirm your appointments as Chairperson of the Department of Physiology and Director of the Cardiovascular Research Center (CVRC), effective July 1, 2011.

Your appointments as Chairperson and Center Director are administrative appointments made by the Dean of the School of Medicine and therefore, you serve in this position at the pleasure of the Dean. These appointments will begin on July 1, 2011 and will end on June 30, 2017 unless otherwise renewed.

Your responsibilities and duties as Chairperson will include, but are not limited to:

- Administering all educational, scholarly and fiscal activities within the Department;
- Overseeing all departmental educational activities, striving for excellence in all programs, and adhering to the policies and guidelines of the department, school, university, and accrediting bodies;
- Enhancing the peer-reviewed research performed by members of the department reflected in peer-reviewed publications:
    - sustaining and improving the number and quality of extramural scientific presentations by members of the Department;
    - developing collaborative relationships with other research programs and Centers in the School and/or University; and
    - meeting or exceeding School targets for research productivity, including extramural salary support and grant expenditure per square foot benchmarks;
- Mentoring junior, mid-level and senior faculty members to support their professional success at all stages of their career;
- Reviewing and recommending faculty and staff for appointment, reappointment, promotion, tenure, and compensation changes, evaluating combined performance in teaching, research, and service and following School/University guidelines and criteria;
- Conducting annual progress reviews with each faculty member and setting his/her goals and objectives for the coming year(s);
- Carrying out periodic program reviews as required by the School, University and accrediting bodies;

- Representing the Department by participation on college and university committees as appropriate: and
- Promoting a strong culture of institutional stewardship, including a commitment to excellence, efficient and effective business operations, efficient and effective use of space and a willingness to re-align resources with evolving strategies:
    - identifying resource opportunities, including philanthropy, to support Department needs;
    - ensuring financial stability by aligning expenses with resources and investment priorities. Managing expenditures to revenues and budget, without deficits; and
    - ensuring compliance with all regulations of the Center, School, University and external funding agencies, including time and effort reporting.

Your responsibilities and duties as Director will include, but are not limited to:

- Fostering the research mission of the Center:
    - developing, presenting for approval, and executing a strategic plan for the Center;
    - developing strong interdisciplinary research collaboration with investigators in the School of Medicine and other schools and research centers in the University;
    - recruiting strong investigators to the Center with high levels of peer-reviewed externally funded research, or the ability to develop same;
    - ensuring that the research effort of your research faculty is supported by external sources, and incentivizing your faculty to increase grant salary support;
    - meeting or exceeding School targets for research productivity, including extramural salary support and grant expenditure per square foot benchmarks;
    - ensuring high levels of scholarly productivity by Center faculty, as reflected in publications in peer-reviewed journals and invited presentations;
    - mentoring research faculty and personnel;
- Providing oversight of teaching programs for students and postdoctoral fellows that are sponsored or participated in by faculty members of the Center.;
- Reviewing and recommending faculty and staff for appointment, reappointment, promotion, and compensation changes, following School/University guidelines and criteria;
- Carrying out periodic program reviews as required by the School, University and accrediting bodies;
- Promoting a strong culture of institutional stewardship, including a commitment to excellence, efficient and effective business operations, efficient and effective use of space and a willingness to re-align resources with evolving strategies;
    - Identifying resource opportunities, including philanthropy, to support Center needs;
    - ensuring financial stability by aligning expenses with resources and investment priorities;
    - ensuring compliance with all regulations of the Center, School, University and external funding agencies, including time and effort reporting;

- Representing the Center by participation on college and university committees as appropriate.

These responsibilities may be amended from time to time by the Dean or University Administration.

Your compensation for the period April 1, 2012 through June 30, 2013 will be as follows:

[redacted]

Redacted

As of July 1, 2013, your B Component will be determined using the extramural grant salary support formula in place at that time. As of July 1, 2013 your stipends as Chair and Center Director will be dependent on your accomplishment of goals established for both the Physiology Department and CVRC. Please submit your proposed goals/expectations for each unit, which at a minimum will reflect meeting the School's targets for research productivity, to Executive Dean Arthur Feldman no later than May 31, 2012.

Please confirm that you are willing to serve as Chairperson and Center Director by signing a copy of this letter and returning it to my office by Wednesday, March 28, 2012. If we have not received your signed acceptance by that date, we will assume that you have not accepted this offer.

We know you will continue to make important contributions to the School of Medicine, and are very pleased you are willing to continue to assume these important responsibilities.

Sincerely,

Larry R. Kaiser, MD
CEO, Temple University Health System
Senior Executive Vice President for Health Affairs
Dean, Temple University School of Medicine

Arthur Feldman, MD, PhD
Executive Dean, Temple University School of Medicine
Chief Academic Officer, Temple University Health System

I confirm my willingness to serve as Chairperson of the Department of Physiology and Director of the Cardiovascular Research Center and to assume the duties as outlined in this letter.

Steven R. Houser

Steven R. Houser, PhD

3/20/2012
Date

EXHIBIT "B"

TEMPLE UNIVERSITY
Lewis Katz School of Medicine

Larry R. Kaiser, M.D., Dean
Senior Executive V.P., Health Affairs
President and CEO,
Temple University Health System

3500 N Broad Street
Medical Education Research Building
Suite 1141
Philadelphia, PA 19140

phone: 215-707-8773
fax: 215-707-8431
E-mail: larry.kaiser@tuhs.temple.edu
web: www.temple.edu/medicine

March 23, 2017

Steven Houser, PhD
1168 Kenyon Drive
Fort Washington, PA 19034-1628

Dear Dr. Houser:

I am pleased to offer you renewal of your appointments as Senior Associate Dean, Research (SADR); Chair of the Department of Physiology, and Director of the Cardiovascular Research Center (CVRC) in the School of Medicine for the period January 1, 2017 to June 30, 2022. These appointments are administrative appointments made by the Dean of the School of Medicine and therefore you serve in these positions at the pleasure of the Dean. These appointments will end on June 30, 2022 unless otherwise renewed.

Compensation
Effective January 1, 2017, your annual stipends for these administrative appointments will be as follows, for the period of time you serve in the roles described:

[redacted] Redacted

Should you relinquish the Chair and/or Director roles during the renewal period noted above at my direction as part of succession planning and/or a restructuring of basic science departments and research centers in the School, and you continue to retain the SADR role, your SADR stipend will increase by the amount of the Chair and/or Director stipends noted above.

The stipends noted above are in addition to your A component compensation and any B component compensation for which you may be eligible. You will continue to be eligible for salary increments and incentive pay based on your score in the annual basic science faculty performance matrix. Your B component for the fiscal year periods July 1, 2017 through June 30, 2022 will be calculated annually according to the School's formula in place in those fiscal years.

Senior Associate Dean, Research (SADR) Responsibilities

Your responsibilities and duties as SADR include, but are not limited to:

- **Providing vision, leadership, strategic development and oversight of all aspects of the School of Medicine's research enterprise.**
- **Leading ongoing research strategic planning and evaluation processes, including but not limited to:**
  - **Preparation of annual evaluation reports of School's progress on meeting goals of existing research strategic plan;**
  - **Update of School's research strategic plan at least every four years;**
  - **Regular program reviews of existing research centers, development of proposals for new research centers (as funding permits) and/or consolidation of research centers;**
  - **Regular review of internal indirect cost recovery (ICR) rates for principal investigators (PI), units and the School, including impact on the School budget and utilization/investment of PI, unit and school fund balances. This includes garnering feedback from all stakeholders and communicating any changes effectively.**
  - **Together with the Senior Associate Deans for Faculty Affairs and Education, School Finance/Administration and University academic leadership, develop framework and potential communication campaign for consolidation of basic science academic departments;**
  - **Preparing a formal, comprehensive review of the School's clinical research administrative structure and outcomes, with recommendations for changes that will improve the School's clinical research funding ratio (vs submissions and expense structure);**
  - **Development of research collaboration with other University schools and colleges; and**
  - **Development of need analysis, plans and funding mechanisms for additional research space, as appropriate.**
- **Overseeing School research administration in all aspects, including but not limited to:**
  - **Compliance with sponsor, University and School policies;**
  - **Resolution of issues between/among PIs, units and/or administrative staff;**
  - **Approval of exceptions to school policies and OVPR deadlines as appropriate,**
  - **Investigation and/or reporting of research integrity issues;**
  - **Oversight of internal funding awards, including recommendations for research focus areas for internal awards and faculty awardees for both School and OVPR internal awards;**
  - **Leadership in advocating with OVPR for process improvements in pre-award and post-award management of sponsored programs; and**
  - **Oversight of research administration organizational structure.**
- **Developing effective working relationships with University research leadership.**
- **Participating actively in peer groups sponsored by national professional organizations such as the American Association of Medical Colleges and establish dialogues with leaders of the research enterprise activities at other medical schools.**

Chair Responsibilities

Your responsibilities and duties as Chair include, but are not limited to:

- Administering all educational, scholarly and fiscal activities within the Department.
- Overseeing all departmental educational activities, striving for excellence in all programs, and adhering to the policies and guidelines of the department, school, university, and accrediting bodies.
- Enhancing the peer-reviewed research performed by members of the department reflected in peer-reviewed publications:
    - Sustaining and improving the number and quality of extramural scientific presentations by members of the Department;
    - Developing collaborative relationships with other research programs and Centers in the School and/or University; and
    - Meeting or exceeding School targets for research productivity, including extramural salary support and grant expenditure per square foot benchmarks.
- Mentoring junior, mid-level and senior faculty members to support their professional success at all stages of their career.
- Reviewing and recommending faculty and staff for appointment, reappointment, promotion, tenure, and compensation changes, evaluating combined performance in teaching, research, and service and following School/University guidelines and criteria.
- Conducting annual progress reviews with each faculty member and setting his/her goals and objectives for the coming year(s).
- Carrying out periodic program reviews as required by the School, University and accrediting bodies.
- Representing the Department by participation on college and university committees as appropriate.
- Promoting a strong culture of institutional stewardship, including a commitment to excellence, efficient and effective business operations, efficient and effective use of space and a willingness to re-align resources with evolving strategies:
    - Identifying resource opportunities, including philanthropy, to support Department needs;
    - Ensuring financial stability by aligning expenses with resources and investment priorities. Managing expenditures to revenues and budget, without deficits; and
    - Ensuring compliance with all regulations of the Center, School, University and external funding agencies, including time and effort reporting.

Director Responsibilities

Your responsibilities and duties as Director include, but are not limited to:

- Fostering the research mission of the Center.
    - Developing, presenting for approval, and executing a strategic plan for the Center;

- Developing strong interdisciplinary research collaboration with investigators in the School of Medicine and other schools and research centers in the University;
- Recruiting strong investigators to the Center with high levels of peer-reviewed externally funded research, or the ability to develop same;
- Ensuring that the research effort of your research faculty is supported by external sources, and incentivizing your faculty to increase grant salary support;
- Ensuring high levels of scholarly productivity by Center faculty, as reflected in publications in peer-reviewed journals and invited presentations; and
- Mentoring research faculty and personnel.

- Providing oversight of teaching programs for students and postdoctoral fellows that are sponsored or participated in by faculty members of the Center.
- Reviewing and recommending faculty and staff for appointment, reappointment, promotion, tenure, and compensation changes, following School/University guidelines and criteria.
- Carrying out periodic program reviews as required by the School, University and accrediting bodies.
- Promoting a strong culture of institutional stewardship, including a commitment to excellence, efficient and effective business operations, efficient and effective use of space and a willingness to re-align resources with evolving strategies:
  - Identifying resource opportunities, including philanthropy, to support Center needs;
  - Ensuring financial stability by aligning expenses with resources and investment priorities; and
  - Ensuring compliance with all regulations of the Center, School, University and external funding agencies, including time and effort reporting.
- Representing the Center by participation on college and university committees as appropriate.

The above responsibilities may be amended from time to time by the Dean.

If, as I hope, you find this offer to be satisfactory as presented, please indicate your acceptance by signing and dating the acknowledgement on the last page and returning a copy of the entire letter by fax or email to Bryant Tabb, Director, Office of Faculty Affairs, at btabb@temple.edu or via fax number 215-707-4659 by Monday, April 3, 2017. If we have not received your signed acceptance by that date, we will assume that you have not accepted this offer. If you require any additional time to consider this offer, please contact Mr. Tabb either by email or phone 215-707-0163; Mr. Tabb will confirm any extension in writing.

I look forward to your continued leadership of the research enterprise of the School, the Department of Physiology and CVRC.

Sincerely,

Larry R. Kaiser, MD
Dean, Lewis Katz School of Medicine
Senior Executive V.P., Health Sciences
President and CEO, Temple University Health System

cc: Kevin Delaney, Vice Provost for Faculty Affairs
Sharon Boyle, Associate Vice President, Human Resources
Ann Untalan, Assistant Dean, Finance

I have reviewed and understand the terms and conditions of appointment as outlined above. By signing this Appointment Letter I agree to all of those terms.

Steven R Houser
(Signature)

3/23/17.
(Date)

EXHIBIT "C"

Heart Fail Rev (2015) 20:423–434
DOI 10.1007/s10741-015-9487-6

BAG3: a new player in the heart failure paradigm

Tijana Knezevic[1] · Valerie D. Myers[2] · Jennifer Gordon[1] · Douglas G. Tilley[3] · Thomas E. Sharp III[2] · JuFang Wang[4] · Kamel Khalili[1] · Joseph Y. Cheung[4] · Arthur M. Feldman[2,4]

Published online: 30 April 2015

Abstract BAG3 is a cellular protein that is expressed predominantly in skeletal and cardiac muscle but can also be found in the brain and in the peripheral nervous system. BAG3 functions in the cell include: serving as a co-chaperone with members of the heat-shock protein family of proteins to facilitate the removal of misfolded and degraded proteins, inhibiting apoptosis by interacting with Bcl2 and maintaining the structural integrity of the Z-disk in muscle by binding with CapZ. The importance of BAG3 in the homeostasis of myocytes and its role in the development of heart failure was evidenced by the finding that single allelic mutations in BAG3 were associated with familial dilated cardiomyopathy. Furthermore, significant decreases in the level of BAG3 have been found in end-stage failing human heart and in animal models of heart failure including mice with heart failure secondary to transaortic banding and in pigs after myocardial infarction. Thus, it becomes relevant to understand the cellular biology and molecular regulation of BAG3 expression in order to design new therapies for the treatment of patients with both hereditary and non-hereditary forms of dilated cardiomyopathy.

Keywords BAG3 · Apoptosis · Autophagy

Introduction

Bcl2-associated athanogene 3 (BAG3) is a 575 amino acid anti-apoptotic protein that is constitutively expressed in the heart, skeletal muscle and some cancers and serves as a co-chaperone of both the constitutively and non-constitutively expressed heat-shock proteins (Hsc/Hsp) [1, 2] (Fig. 1). When bound to Hsp's, BAG3 plays a critical function in regulating protein quality control (PQC) [2] and by interacting with Bcl2, it protects cells from apoptotic death [3]. The BAG3-HSP protein–protein interaction is increasingly recognized as a therapeutic target in the treatment of cancer [4, 5]. Recently, it has been shown that BAG3 plays a role in the stability of the cardiac sarcomere through regulation of filamin clearance and production and by binding to CapZ [6] (Fig. 2). Two seminal findings led to the recognition that BAG3 could play a substantive role in the development of or progression of heart failure. First, Homma showed that mice with homozygous disruption of BAG3 developed a fulminant myopathy characterized by non-inflammatory myofibrillar degeneration, disruption of Z-disk architecture, apoptotic features in the early postnatal period and death by 4 weeks of age [7]. Second, Selcen reported three children with myofibrillar myopathy who harbored a

Tijana Knezevic and Valerie D. Myers have contributed equally to this manuscript.

✉ Arthur M. Feldman
arthur.feldman@tuhs.temple.edu

1 Department of Neuroscience, Temple University School of Medicine, 3500 N. Broad Street, Suite 1150, Philadelphia, PA 19140, USA

2 Department of Physiology, Temple University School of Medicine, 3500 N. Broad Street, Suite 1150, Philadelphia, PA 19140, USA

3 Department of Pharmacology, Temple University School of Medicine, 3500 N. Broad Street, Suite 1150, Philadelphia, PA 19140, USA

4 Department of Medicine, Temple University School of Medicine, 3500 N. Broad Street, Suite 1150, Philadelphia, PA 19140, USA

Fig. 1 BAG3 protein adapted from McCollum et al. [118]

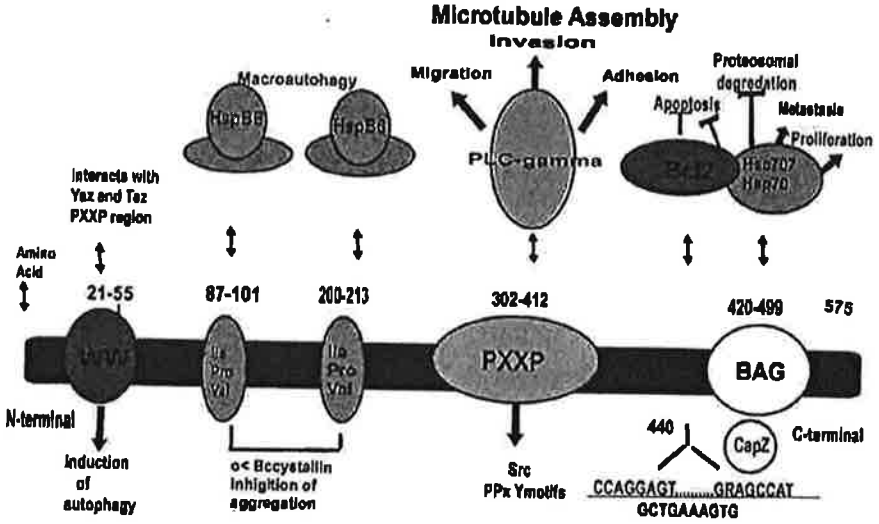
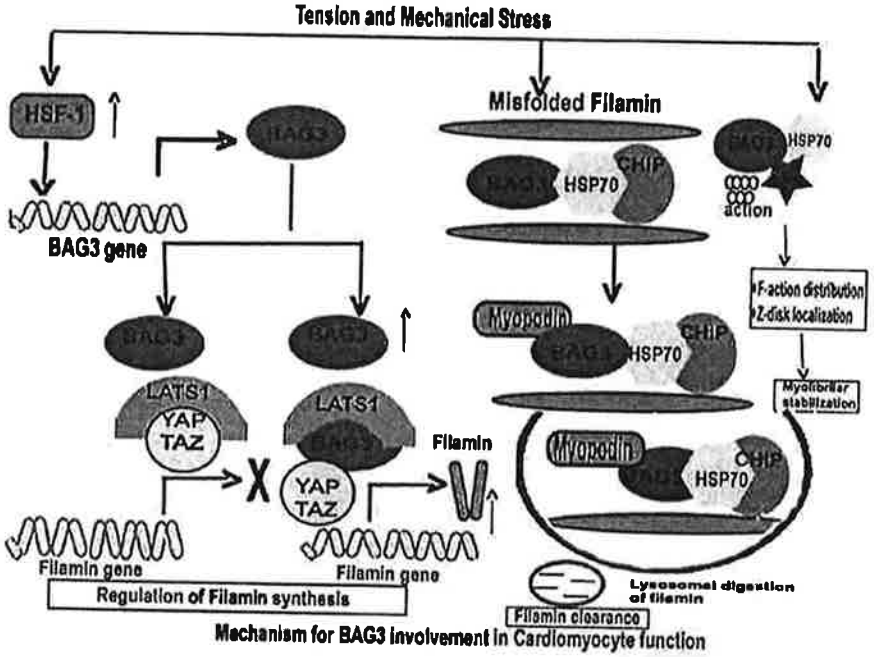


Fig. 2 Role of BAG3 in the cell



single allelic substitution of BAG3 [8]. In addition, knockdown of BAG3 in zebrafish [9] or in neonatal cardiomyocytes [10] leads to significant cardiac dysfunction. Thus, BAG3 appears to be an exciting new target for therapeutic intervention in patients with heart failure. Here, we review our current understanding of the biology and pathobiology of BAG3 as it relates to the heart.

BAG3 mutants, myofibrillar myopathy and dilated cardiomyopathy

The first evidence that BAG3 could play an important role in the pathobiology of the heart came from a study by Homma which demonstrated that mice in which BAG3 had been knocked out had non-inflammatory myofibrillar

Heart Fail Rev (2015) 20:423–434

degeneration, disruption of Z-disk architecture, apoptotic features in the early postnatal period and death by 4 weeks of age [7]. However, it was the finding that mutations in BAG3 were associated with the development of muscle disease in children that led investigators to propose that changes in BAG3 function could result in the development of left ventricular dysfunction and heart failure. Selcen and co-workers first reported three children with myofibrillar myopathy who harbored a single allelic substitution of Leucine (Leu) for Proline (Pro) at position 209 (exon 3 of BAG3) of BAG3 [8]. All three patients presented in childhood with progressive muscle weakness, respiratory insufficiency and cardiac dilatation with systolic dysfunction. The parents were asymptomatic and did not have the Pro209Leu genotype. Odgerel et al. [11] reported an additional three families with the same BAG3 p.Pro209Leu genotype, but the severe myofibrillar myopathy in these patients was accompanied by axonal neuropathy with giant axons. One asymptomatic parent showed somatic mosaicism, whereas in the other two families, the parents had a normal genotype supporting the observation by Selcen that spontaneous mutations could occur. In this group of patients, early respiratory failure was more common than heart failure [12].

The first suggestion that BAG3 could play a role in adult-onset familial dilated cardiomyopathy came from a study of patients with a dilated cardiomyopathy, diffuse myocardial fibrosis and sudden death. The phenotype was associated with a locus on chromosome 10q2-26, a region that included the BAG3 gene [13]. Two mutations in BAG3 were subsequently identified in Japanese patients with familial dilated cardiomyopathy (Arg218Trp and Leu462Pro). When these mutations were expressed in neonatal rat cardiomyocytes, functional studies showed impaired Z-disk assembly and increased sensitivity to stress-induced apoptosis [14]. Norton et al. [9] identified a deletion of BAG3 exon 4 as causative of familial dilated cardiomyopathy in a family without neuropathy or peripheral muscle weakness. Zebrafish expressing this mutation demonstrated cardiac enlargement and hypertrophy. Subsequent sequencing of BAG3 in subjects diagnosed with idiopathic dilated cardiomyopathy (IDC) identified four additional mutations that segregated with all relatives affected by the disease. A genome-wide association study (GWAS) conducted in patients with HF secondary to IDC implicated a non-synonymous single nucleotide polymorphism (SNP) (c.757T > C, [p. Cys151Arg]) located within the BAG3 gene as contributing to sporadic dilated cardiomyopathy [15]. More recently, we found a 10 nucleotide mutation in exon 6 of the BAG3 gene in a large family with familial dilated cardiomyopathy [16]. The mutation segregated with all affected family members and predicted a shift in the reading frame that would result in the deletion of 135 amino acids from the C-terminal end of the protein that encompassed a large portion of the BAG region [16].

Interestingly, a western blot of protein extracted from the left ventricular myocardium of a family member who underwent heart transplantation demonstrated a level of BAG3 in the heart that was less than half of that seen in non-failing control hearts obtained from organ donors whose hearts could not be used for transplant because of blood type or size incompatibility. However, our finding that levels of BAG3 were also diminished by nearly 50 % in hearts from patients with end-stage heart failure undergoing cardiac transplantation that had a normal BAG3 genotype led us to propose that deficiencies in BAG3 might be a critical component in the progression of heart failure humans [16]. Indeed, as seen in Fig. 3, we have also found that mice with severe heart failure 18 weeks after trans-aortic banding demonstrate significant decreases in BAG3 levels that are comparable to the decrease seen in patients with heart failure. Similarly, pigs with HF secondary to occlusion of the left anterior descending coronary artery (Fig. 4a–d) also demonstrated significant reductions in levels of BAG3 (Fig. 4e, f). The decrease in BAG3 in humans, pigs and mice with heart failure was not associated with a change in the levels of BAG3 mRNA suggesting that posttranslational modifications account for the decrease. A recent report demonstrates that BAG3 levels are increased in the sera of patients with HF [17]. The same group also reported increased levels of BAG3 antibodies in the sera of patients with HF [18]. However, by contrast with most biomarkers including BNP and TNFx [19], BAG3 levels were only decreased in patients with NYHA Class IV HF, although these results will need to be confirmed in a larger group of patients.

Genetic heterogeneity is a common feature of genetic mutations in cardiac genes, and thus, it is not surprising that individuals with mutations in BAG3 can present with a variety of cardiovascular phenotypes. For example, in the family that we reported, the onset of symptomatic heart failure occurred as early as 18 years of age and as late as 48 years of age. BAG3 mutations can also be associated with a diverse set of phenotypes. For example, a Chinese patient with restrictive lung disease, a rapidly progressive proximal myopathy, rigid spine, bilateral Achilles tendon tightening, hypertrophic cardiomyopathy with restrictive physiology and a prolonged QT interval had de novo mutation at c.626C > T (p.Pro209Leu) which is situated in the conserved Ile-Pro-Val motif that is a site of interaction between BAG3 and the Hsps as well as a second non-synonymous change c.772C > T (p.Arg258Trp) which was not situated in a known structural domain [20]. Interestingly, polymorphisms of BAG3 may also play a role in the pathogenesis of tako-tsubo cardiomyopathy [21].

Structure–function relationships of BAG3

A partial sequence of BAG3 protein was first isolated in 1999 using a yeast two-hybrid screen with Hsp70 as bait

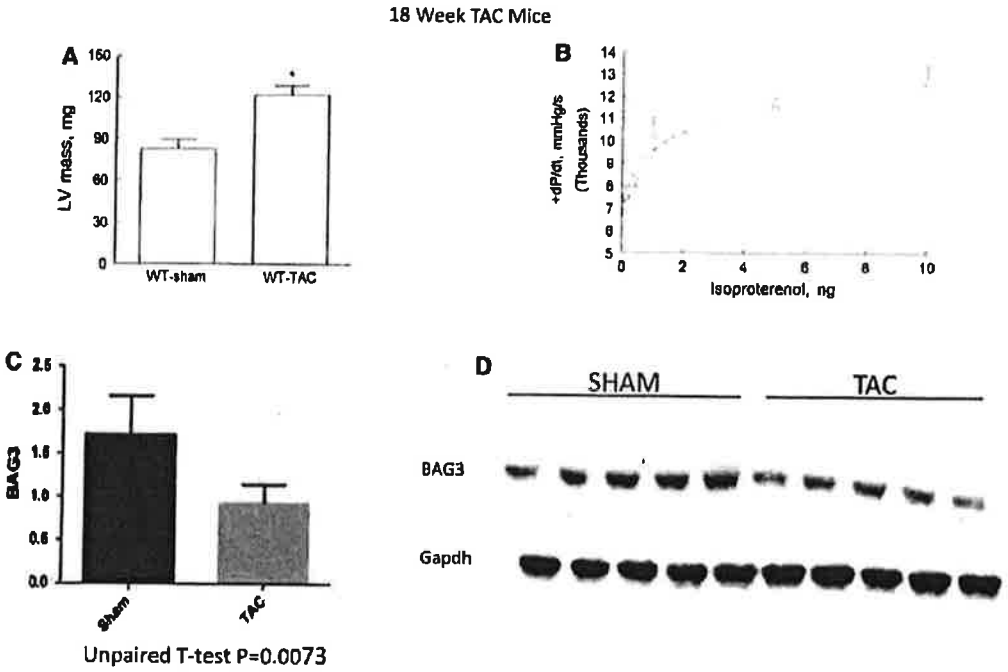


Fig. 3 BAG3 levels in failing murine hearts. Wild-type c57BL/6 mice underwent trans-aortic banding (TAC) as has been described previously [119]. Eighteen weeks after TAC, *left ventricular* contractility was measured using a conductance catheter inserted into the *left ventricle* through a carotid approach as described previously. Heart weight to body weight ratios were calculated after killing (**a**). Contractility was measured during intravenous infusion of increasing doses of catecholamine (**b**). **b** Sham-operated hatched line = control; solid line = TAC mice. Hearts were then frozen for subsequent measurement of BAG3 levels. Myocardial proteins were extracted as we have described previously separated by gel electrophoresis and probed with a murine BAG3 antibody. As seen in **c**, there was a significant decrease in BAG3 levels by western blotting in TAC mice when compared with sham-operated controls. A representative western blot is seen in **d**

[22] (Fig. 1). Investigators cloned BAG3 from cDNA libraries using recombinant Bcl2 [23, 24]. BAG3 is highly conserved in nature at both the gene and protein level with significant homology across mice, pigs and humans [25]. By contrast, BAG3 has little in common with the other members of the BAG family with the exception that all members of the family share a common BAG domain. Located at the C-terminal end of the protein, this region consists of three alpha helices of 30–40 amino acids each that bind to a motif in the ATPase domain of Hsp70, to Bcl2 and to small heat-shock proteins (HspX or sHsp) [26]. The length of the BAG domains varies with two distinct forms: A 'long' BAG domain that is specific for BAG1 and a 'short' domain that is found in BAG3, BAG4 and BAG5 [27]. Only BAG1 and BAG3 interact with Bcl2, and BAG4 is physiologically distinguishable from the other BAG family members in that it blocks TNF receptor signaling [24, 28–32]. BAG6 (Scythe) regulates nuclear pathways and cytochrome c release [33, 34]. BAG3 also contains a WW domain near its N-terminal region [35] and a PXXP domain that binds phospholipase Cγ-1 [36]. The WW domain and the PXXP domain may also connect BAG3 to the SH3 domain of Src thereby mediating the effects of Hsp70 on Src signaling and to PPxY motifs of signaling proteins providing a platform for the assembly of multi-protein networks [37]. BAG3 also binds to αB-crystallin via a highly conserved intermediate domain (Ile-Pro-Val) that facilitates its ability to inhibit protein aggregation [38].

Studies using Htt43Q, a pathogenic form of huntingtin that is responsible for Huntington's disease, as a molecular probe have helped to define the role of some of the motifs found in BAG3. The BAG domain is required for interaction with Hsp70 and Bcl-2 but not with HspB8 (HspB8 and HspB6 bind to IPV domains) yet BAG3 is able to clear huntingtin even in the absence of the BAG region [39, 40]. By contrast, deletion of the WW domain had no effect on Hsp70, Bcl-2 or HspB8 binding and had no effect on Htt43Q degradation. Deletion of the proline-rich PXXP region also did not alter Hsp70, Bcl-2 or HspB8 binding—but abrogated the ability of the cell to clear Htt43Q [40]. The only protein known to interact with the PXXP proline-rich region of BAG3 is phospholipase Cγ-1 which

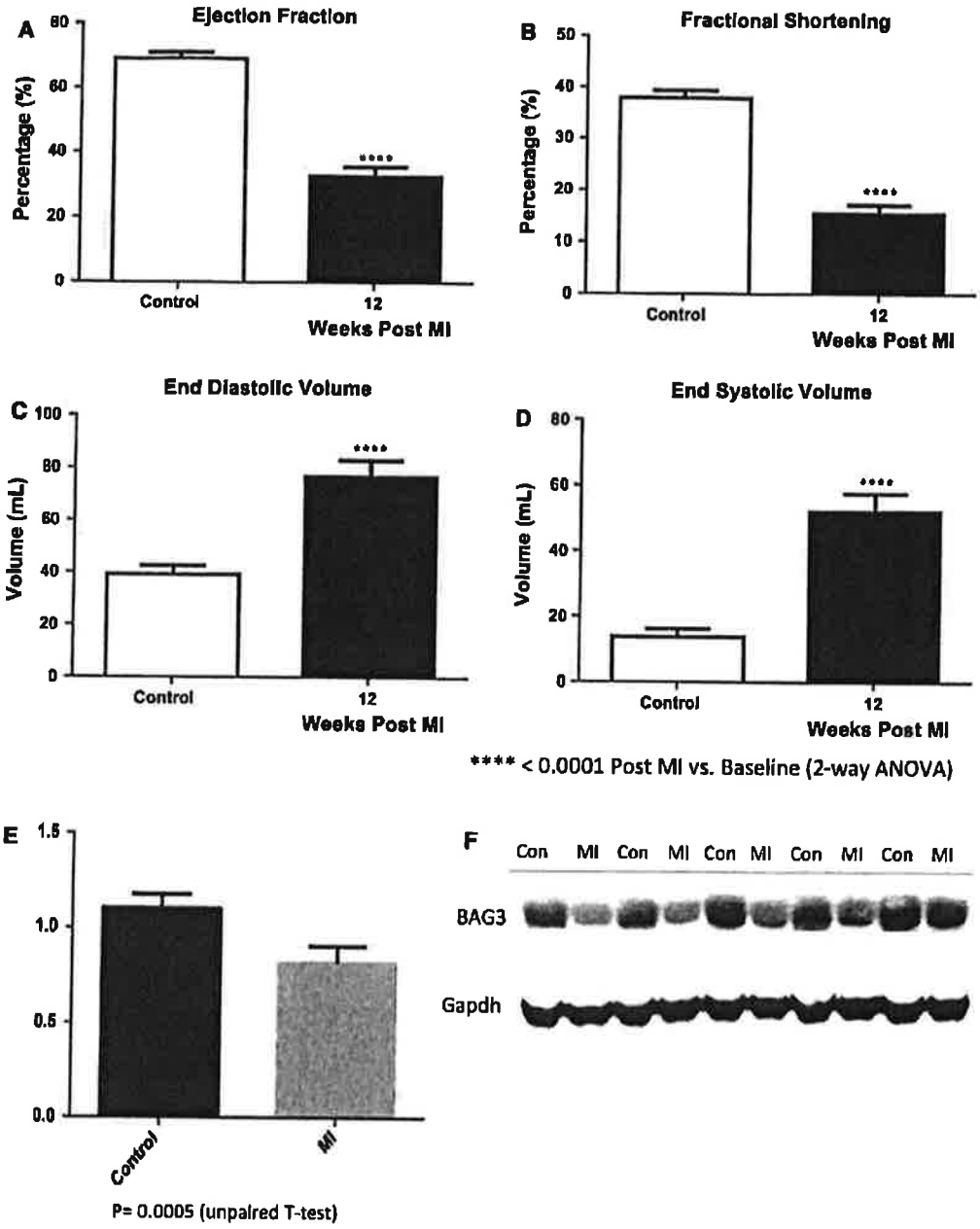


Fig. 4 Hemodynamic indices and BAG3 levels in non-infarcted *left ventricular* myocardium from a pig 4 weeks after balloon occlusion of the *left anterior* descending coronary artery. **a** ejection fraction; **b** fractional shortening; **c** end diastolic volume; **d** end systolic volume; **e** BAG3 levels; **f** representative western blot

modulates microtubule assembly [36, 41]. However, PKCγ-1 knockdown had no affect on the ability of BAG3 to clear Htt43Q. Taken together, these results suggest that HspB8 plays an important role in the clearance of mutated proteins such as Htt43Q; however, the specific role of the PXXP region remains to be defined.

Regulation of BAG3 expression

BAG3 expression is increased by the stress associated with heavy metals, high temperature [42, 43] oxidants [44], proteasome inhibitors [45], serum starvation [46] light damage in the retina [47]; seizure activity [48];

hemodialysis [49]; hypoxia [50]; and HIV infection [51]. In addition, BAG3 expression is increased in a large number of cancers including: acute lymphocytic and B cell chronic lymphocytic leukemia [52, 53] thyroid carcinoma [54]; melanomas [55] non-small-cell lung cancer [56]; hepatocellular carcinoma [57]; pancreatic adenocarcinoma [58], small cell carcinoma of the lung [59] and glioblastoma [60]. The overexpression of BAG3 in malignant cells increased motility and metastasis, whereas reduction in BAG3 protein by RNA interference decreased cell motility. Cells from BAG3-deficient mice showed delayed formation of filopodia and focal adhesion complexes—putatively mediated by decreased activity of the small GTPase Rac1 that is involved in actin cytoskeleton dynamics. Furthermore, mice with reduced BAG3 showed suppressed invasion and metastasis of a human tumor xenograft [61]. Consistent with the finding that BAG3 is involved in cell adhesion, motility and metastasis of cancer cells, Franco et al. [55] demonstrated that melanoma tumors that have metastasized to distant organs had high levels of BAG3 expression. Interestingly, BAG3 is able to modulate its own transcription through a positive feedback loop involving its 5′-untranslated region (UTR) sequence—a process that is mediated by the BAG domain but is independent of BAG3 association with the UTR sequence [62]. This ability to self-regulate in a positive manner may account for the long-term survival of malignant cells.

The predominant mechanism through which stress increases BAG3 expression is induction of heat-shock factor 1 (HSF1) [63]. Stress causes induction of both HSF1 and the HSF target gene DNAJB1 in smooth muscle although the predominant factors regulating BAG3 expression in the heart have not been defined [64]. WT1, an oncogene that is expressed in a variety of tumors and that is associated with a poor response to therapy also induces BAG3 expression by binding to sequences in the promoter region of BAG3 [65, 66]. By contrast, serum starvation downregulates BAG3 expression at the transcriptional level via c-Jun [46]. BAG3 expression is also regulated by the transcription factors Egr1 and AibZIP [67, 68]. Importantly, BAG3 function and levels can also be regulated by posttranslational modification. For example, phosphorylation of Ser187 of BAG3 by protein kinase C delta (PKCΔ) leads to increased epithelial-mesenchymal transition, motility and invasiveness of cancer cells [69, 70]. Expression of exogenous Tat, a protein expressed by the HIV virus, in glioblastoma cell lines enhances BAG3 protein but not mRNA levels [71]. BAG3 levels are also increased by drugs including: TNF-related apoptosis-inducing ligand, fludarabine, cytosine arabinoside and etoposide [1, 45, 54, 72]. Proteasome inhibitors induce a BAG3-dependent noncanonical autophagy in HepG2 cells although the specific mechanism for this effect has not been elucidated [73]. BAG3 levels are also enhanced by decreased calcium influx as caused by exposure to carboxyamido-triazole (CAI) an inhibitor of non-voltage-gated calcium channels [36]. The physiologic significance of this finding in cardiomyocytes is under investigation in our own laboratories. Both JNK and NF-kB induce BAG3 expression in the presence of lipopolysaccharide suggesting that BAG3 is also responsive to the stress associated with enhanced expression of pro-inflammatory cytokines and therefore may participate in inflammatory diseases including that of the heart [74]. The p38 MAP kinase increases BAG3 transcription in HeLa cells exposed to oxidative stress, although the presence of this pathway in cardiac cells has not be elucidated [75].

Autophagy and apoptosis

All eukaryotic cells depend on the presence of a system for protein quality control (PQC). PQC acts as a surveillance system that assures proper protein folding as well as recognition of misfolded and dysfunctional proteins or protein aggregates and initiates protein refolding or clearance. BAG3 plays a critical role in this process (Fig. 2). PQC relies on molecular chaperons and co-chaperons that can sense misfolded proteins and then either initiate refolding or elimination of the folded or damaged proteins from the cell. Eukaryotic cells have two major intracellular protein degradation pathways, ubiquitin–proteasome and autophagy-lysosome systems. The ubiquitin–proteasome system (UPS) is composed of a barrel-shaped protein complex with a 13-A wide opening through which ubiquinated misfolded proteins have to pass in order to be degraded into smaller reusable peptides [76]. Some protein aggregates are too large to fit into the 13-A wide channel of the proteasome. These large aggregates are degraded by the aggresome-autophagy system. Chaperones and co-chaperones can participate in both of these systems by identifying selective proteins for destruction. In fact, the BAG family of proteins can regulate whether misfolded proteins are degraded by the proteasomal or by the autophagy pathways. Autophagy systems can be divided into macroautophagy, microautophagy and chaperone-mediated autophagy [77] (Figs. 1, 2).

In a multi-step process, macroautophagy sequesters protein aggregates in autophagasomes, double-layered membrane structures found in the cytoplasm. The protein aggregates are then transported to and fused with the lysosome for degradation by lysosomal hydrolases [78]. In microautophagy, the cargo enters lysosomes directly by invagination of the lysosomal membrane resulting in degradation of the aggregated protein content by lysosomal enzymes. Macroautophagy is a somewhat promiscuous

system; however, recent studies have shown that BAG3 also participates in selective macroautophagy that is responsible for homeostatic regulation of specific proteins. When these clearance mechanisms become overwhelmed in neuronal cells, increased levels of abnormal protein aggregates can lead to the progression of a number of neurologic diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and spinocerebellar ataxia type 3 [79–82]. Indeed, overexpression of BAG3 induced decreases in tau, a protein that plays a fundamental role in the pathogenesis of Alzheimer's disease [83].

In selective macroautophagy, BAG3 is coupled with the chaperone Hsp70 and the co-chaperone ubiquitin ligase carboxyl terminal of Hsp70/Hsp90 interacting protein (CHIP) and facilitates the sequestration of misfolded proteins into autophagasomes [84]. Investigators have recently focused their interest on two IPV (Ile-Pro-Val) motifs in BAG3 that regulate its stoichiometric interaction with the small heat-shock proteins (sHsp) sHsp6 and sHsp8 [40, 85, 86]. The multi-chaperone complex of BAG3-HspB8-Hsp70 can selectively cause misfolded proteins to be degraded by macroautophagy—a function that may require the cooperation of the macroautophagy receptor protein p62/SQSTMI. These proteins in concert can bind simultaneously to ubiquitin and the autophagasome membrane-associated protein LC3 [85, 87–90]. Once coupled to the chaperone and co-chaperone complexes, misfolded proteins as well as autophagic vacuoles are transported retrograde along cytoskeletal tracks by ATP-fueled motor proteins called dynein motor proteins to perinuclear microtubule organizing centers or MTO's. Once there, they are packaged in protein structures called aggresomes for eventual disposal or engulfed and degraded by the autophagic vacuoles [91–94]. Recently, Gamerdinger and colleagues have reported that BAG3 mediates the transport of proteins to the aggresome by catalyzing substrate transfer from Hsp70 to the dynein motor complex [78, 89].

A second major role for BAG3 is that it inhibits apoptosis through multiple mechanisms—many of which have been elucidated in cancer cells. Multiple forms of cellular stress and noxious stimuli activate signals that converge into a common pathway that is triggered by caspases [95–97]. The anti-apoptotic members of the Bcl-2 family of proteins (Bcl-2 and Bcl-x) inhibit caspase activation by blocking the release of apoptogenic cytochrome c from the mitochondria and by sequestering the procaspases 8 and 9 [98–104]. BAG3 synergizes with Bcl-2 and with Bcl-XL to protect both normal cells and neoplastic cells from apoptosis [44, 53, 56]. Its overexpression can synergize the anti-apoptotic effect of Bcl-2 [24], whereas BAG3 knockdown increases both basal and drug-induced apoptosis.

BAG3 overexpression can also inhibit apoptosis by modulating the NF-kB pathway [105]. BAG3 mediates the dissociation of the Hsp70- kappa B kinase (IKK-γ: subunit of IKK) complex, which leads to a decrease in Hsp70-mediated delivery of IKK-γ to the proteasome thereby sustaining NF-kB activation and inhibition of cell apoptosis [55]. However, investigators have also reported that NF-kB can modulate the expression of BAG3 as well as the formation of the BAG3-HsB8 complex [106]. Recent studies have suggested a broader role for BAG3. For example, BAG3 regulates epithelial-mesenchymal transition and angiogenesis through ERK phosphorylation [57, 107]; induces epithelial-mesenchymal transition through activation of the transcription factor ZEB1 [108]; and modulates the activity of the transcription factors FoxM1 and Hif1α, the translation regulator HuR and the cell cycle regulators p21 and survivin [109]. BAG3 also downregulates the microRNA-29b which leads to upregulation of the anti-apoptosis protein Mcl-l leading to resistance to anticancer drugs [110].

The molecular mechanisms by which BAG3 modulates the cardiac phenotype

The finding—that mutations in BAG3 were associated with the development of disrupted Z-disk structure, myofibrillar degeneration and disorganization—led Hishiya and colleagues to assess the effects of BAG3 in neonatal rat myocytes [8, 10, 111]. They found that BAG3 insured the structural stability of filamentous actin (F-actin) by promoting association between Hsc70 and the actin capping protein beta 1(CapZβ1). BAG3 also facilitated the cellular localization of CapZβ1. CapZβ1 is a sarcomere protein that: (1) binds with high affinity to the barbed end of actin to prevent its disassociation into actin monomers; (2) interacts with the protein nebulin to position the actin filaments at the Z-disk; (3) links adjacent sarcomeres; and (4) stabilizes the Z-disk [112–114]. BAG3 knockdown led to proteasomal degradation of CapZβ1, whereas inhibition of CapZβ1 led to myofibril disruption in response to mechanical stress. By contrast, overexpression of CapZβ1 prevented myofibril disruption when BAG3 was knocked down [10]. These results were consistent with the finding that mutations in many of the genes encoding Z-disk proteins lead to increased vulnerability to mechanical stress [115, 116], For example, mutations in sarcomere genes such as desmin, αB-crystallin, myotilin, Z-band alternatively spliced PDZ motif containing protein (ZASP) and filamin C result in phenotypes that are very similar to that seen in cells in which BAG3 has been knocked down. Thus, the co-chaperone BAG3 and the chaperone Hsc70 play a critical role in maintaining the

structural integrity of the sarcomere especially during mechanical stress.

Recent studies have shown that BAG3 can also modulate the level of functional filamin, a dimeric actin cross-linker that acts as a signaling hub for various proteins and that also plays an important role in stabilization of the myofibrillar Z-disk [117]. BAG3 removes filamin that has been damaged by mechanical stress through autophagic mechanisms. At the same time, BAG3 stimulates filamin transcription by using its WW domain to engage inhibitors of the transcriptional activators YAP and TAZ [64].

Filamin regulation and clearance and sarcomere stabilization appear to occur in large part through what is now referred to as chaperone-assisted selective autophagy or CASA [6]. CASA differs from macroautophagy described above in that it requires a multi-chaperone complex comprised of a client protein, HspA8-/Hsp70-, HspB8-/Hsp27- and the HspA8-associated ubiquitin ligase STUB/CHIP as well as ubiquitin conjugation enzymes of the UBE2D family. The degradation signal generated by ubiquinitation of the client protein leads to recognition by the autophagic ubiquitin adaptor SQSTM1/p62, autophagasome formation, and protein degradation in lysosomes. This pathway is relevant to muscle as the CASA machinery is localized at the Z-disk, and CASA knockdown leads to disintegration of the Z-disk and resultant pathologic changes in skeletal and cardiac muscle. A predominant feature of CASA is that it is stress related with a predominant substrate of CASA in mechanically stressed cells being the cytoskeletal protein filamin. Thus, in the heart, BAG3 helps rid the myocyte of misfolded and degraded proteins but also maintains the homeostatic balance between filamin breakdown and filamin production.

In adult mouse left ventricular myocytes in which endogenous BAG3 is knocked down by adenovirus-siRNA, we recently observed that systolic calcium concentrations, calcium transient amplitude and single myocyte contraction amplitude are all significantly decreased compared to myocytes infected with adenovirus-GFP (unpublished results). These observations suggest that in addition to regulating CapZB1 and filamin, BAG3 may modulate cardiac contractility by affecting myocyte excitation–contraction coupling.

Conclusion

In summary, BAG3 'chaperones' an array of cellular proteins including the Hsps and the sHsps that play a critical role in maintaining the homeostasis of eukaryotic cells and the balance between autophagy and apoptosis. BAG3 is of particular importance during cell stress as increased apoptotic signals and aggregates of protein debris threaten cell survival. Appropriate levels of BAG3 production and function are of particular importance in the heart because the complex components of the sarcomere are continuously exposed to contractile stretch and strain leading to changes in protein folding and in apoptotic signaling. In addition, BAG3 through binding to CapZ helps to maintain the highly ordered filamentous structure of the Z-disk by clearing filament debris while at the same time stimulating filament synthesis. That BAG3 plays an important role in the progression of heart failure is demonstrated by the finding that loss of function mutations result in the development of both early-onset and late-onset familial dilated cardiomyopathy. However, additional research is required to: (1) elaborate the molecular and cellular mechanisms that account for the decrease in BAG3 levels seen in hearts from patients with end-stage heart failure; (2) identify the effects of cardiac stress and left ventricular dysfunction on the chaperone and co-chaperone peptides that partner with BAG3 including the Hsp's, sHsp's, myopodin and synaptopodin; and (3) assess whether reconstitution of normal levels of BAG3 alone can interrupt the progression of heart failure. Perhaps the most interesting question derives from the fact that while BAG3 expression maintains cell survival by inhibiting apoptosis and by removing the debris that accumulates in cells that are under continuous mechanical tension such as cardiac myocytes, these mechanisms are maladaptive in the presence of malignant cells as increased levels of BAG3 can decrease apoptosis leading to increased tumor growth, enhanced metastasis, decreased sensitivity to chemotherapeutic agents and reduced survival. With BAG3 serving as a new target for chemotherapy, additional studies will be needed to develop approaches that will enhance apoptosis and decrease autophagy in malignant cells while at the same time not influencing these pathways in the heart.

Acknowledgments This work was supported by NIH grant #ROIHL123093. The authors thank Marianne LaRussa for her assistance in preparation of this manuscript.

References

1. Rosati A, Graziano V, De Laurenzi V, Pascale M, Turco MC (2011) Bag3: a multifaceted protein that regulates major cell pathways. Cell Death Dis 2:e141
2. McCollum AK, Casagrande G, Kohn EC (2010) Caught in the middle: the role of bag3 in disease. Biochem J 425:e1–e3

3. Maloyan A, Sayegh J, Osinska H, Chua BH, Robbins J (2010) Manipulation of death pathways in desmin-related cardiomyopathy. Circ Res 106:1524–1532
4. Li X, Colvin T, Rauch JN, Acosta-Alvear D, Kampmann M, Dunyak B, Hann B, Aftab BT, Murnane M, Cho M, Walter P, Weissman JS, Sherman MY, Gestwicki JE (2015) Validation of the hsp70-bag3 protein-protein interaction as a potential therapeutic target in cancer. Mol Cancer Ther. doi:10.1158/1535-7163.MCT-14-0650
5. Sherman MY, Gabai VL (2014) Hsp70 in cancer: back to the future. Oncogene. doi:10.1038/onc.2014.349
6. Ulbricht A, Hohfeld J (2013) Tension-induced autophagy: may the chaperone be with you. Autophagy 9:920–922
7. Homma S, Iwasaki M, Shelton GD, Engvall E, Reed JC, Takayama S (2006) Bag3 deficiency results in fulminant myopathy and early lethality. Am J Pathol 169:761–773
8. Selcen D, Muntoni F, Burton BK, Pegoraro E, Sewry C, Bite AV, Engel AG (2009) Mutation in bag3 causes severe dominant childhood muscular dystrophy. Ann Neurol 65:83–89
9. Norton N, Li D, Rieder MJ, Siegfried JD, Rampersaud E, Zuchner S, Mangos S, Gonzalez-Quintana J, Wang L, McGee S, Reiser J, Martin E, Nickerson DA, Hershberger RE (2011) Genome-wide studies of copy number variation and exome sequencing identify rare variants in bag3 as a cause of dilated cardiomyopathy. Am J Hum Genet 88:273–282
10. Hishiya A, Kitazawa T, Takayama S (2010) Bag3 and hsc70 interact with actin capping protein capz to maintain myofibrillar integrity under mechanical stress. Circ Res 107:1220–1231
11. Odgerel Z, Sarkozy A, Lee HS, McKenna C, Rankin J, Straub V, Lochmuller H, Paola F, D'Amico A, Bertini E, Bushby K, Goldfarb LG (2010) Inheritance patterns and phenotypic features of myofibrillar myopathy associated with a bag3 mutation. Neuromuscul Disord 20:438–442
12. Pfeffer G, Povitz M, Gibson GJ, Chinnery PF (2014) Diagnosis of muscle diseases presenting with early respiratory failure. J Neurol. doi:10.1007/s00415-014-7526-1
13. Ellinor PT, Sasse-Klaassen S, Probst S, Gerull B, Shin JT, Toeppel A, Heuser A, Michely B, Yoerger DM, Song BS, Pilz B, Krings G, Coplin B, Lange PE, Dec GW, Hennies HC, Thierfelder L, MacRae CA (2006) A novel locus for dilated cardiomyopathy, diffuse myocardial fibrosis, and sudden death on chromosome 10q25-26. J Am Coll Cardiol 48:106–111
14. Arimura T, Ishikawa T, Nunoda S, Kawai S, Kimura A (2011) Dilated cardiomyopathy-associated bag3 mutations impair z-disc assembly and enhance sensitivity to apoptosis in cardiomyocytes. Hum Mutat 32:1481–1491
15. Villard E, Perret C, Gary F, Proust C, Dilanian G, Hengstenberg C, Ruppert V, Arbustini E, Wichter T, Germain M, Dubourg O, Tavazzi L, Aumont MC, DeGroote P, Fauchier L, Trochu JN, Gibelin P, Aupetit JF, Stark K, Erdmann J, Hetzer R, Roberts AM, Barton PJ, Regitz-Zagrosek V, Aslam U, Duboscq-Bidot L, Meyborg M, Maisch B, Madeira H, Waldenstrom A, Galve E, Cleland JG, Dorent R, Roizes G, Zeller T, Blankenberg S, Goodall AH, Cook S, Tregouet DA, Tiret L, Isnard R, Komajda M, Charron P, Cambien F (2011) A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy. Eur Heart J 32:1065–1076
16. Feldman AM, Begay RL, Knezevic T, Myers VD, Slavov DB, Zhu W, Gowan K, Graw SL, Jones KL, Tilley DG, Coleman RC, Walinsky P, Cheung JY, Mestroni L, Khalili K, Taylor MR (2014) Decreased levels of bag3 in a family with a rare variant and in idiopathic dilated cardiomyopathy. J Cell Physiol 229:1697–1702
17. De Marco M, D'Auria R, Rosati A, Vitulano G, Gigantino A, Citro R, Piscione F, Zilinski J, Januzzi JL Jr, Turco MC (2014) Bag3 protein in advanced-stage heart failure. JACC Heart Fail 2:673–675
18. De Marco M, Falco A, Basile A, Rosati A, Festa M, d'Avenia M, Pascale M, Dal Piaz F, Bisogni R, Barcaroli D, Coppola G, Piscione F, Gigantino A, Citro R, De Rosa R, Vitulano G, Virtuoso N, Manganelli F, Palermo E, Siano F, Rosato G, Hahne M, Tiberti C, De Laurenzi V, Turco MC (2013) Detection of soluble bag3 and anti-bag3 antibodies in patients with chronic heart failure. Cell Death Dis 4:e495
19. Feldman AM, Mann DL, She L, Bristow MR, Maisel AS, McNamara DM, Walsh R, Lee DL, Wos S, Lang I, Wells G, Drazner MH, Schmedtje JF Jr, Pauly DF, Sueta CA, Di Maio M, Kron IL, Velazquez EJ, Lee KL (2013) Prognostic significance of biomarkers in predicting outcome in patients with coronary artery disease and left ventricular dysfunction: results of the biomarker substudy of the surgical treatment for ischemic heart failure trials. Circ Heart Fail 6:461–472
20. Lee HC, Cherk SW, Chan SK, Wong S, Tong TW, Ho WS, Chan AY, Lee KC, Mak CM (2012) Bag3-related myofibrillar myopathy in a Chinese family. Clin Genet 81:394–398
21. Citro R, d'Avenia M, De Marco M, Giudice R, Mirra M, Ravera A, Silverio A, Farina R, Silvestri F, Gravina P, Villa F, Puca AA, De Windt L, De Laurenzi V, Bossone E, Turco MC, Piscione F (2013) Polymorphisms of the antiapoptotic protein bag3 may play a role in the pathogenesis of tako-tsubo cardiomyopathy. Int J Cardiol 168:1663–1665
22. Takayama S, Xie Z, Reed JC (1999) An evolutionarily conserved family of hsp70/hsc70 molecular chaperone regulators. J Biol Chem 274:781–786
23. Antoku K, Maser RS, Scully WJ Jr, Delach SM, Johnson DE (2001) Isolation of bcl-2 binding proteins that exhibit homology with bag-1 and suppressor of death domains protein. Biochem Biophys Res Commun 286:1003–1010
24. Lee JH, Takahashi T, Yasuhara N, Inazawa J, Kamada S, Tsujimoto Y (1999) Bis, a bcl-2-binding protein that synergizes with bcl-2 in preventing cell death. Oncogene 18:6183–6190
25. Wada S, Hamada M, Satoh N (2006) A genomewide analysis of genes for the heat shock protein 70 chaperone system in the ascidian ciona intestinalis. Cell Stress Chaperon 11:23–33
26. Takayama S, Reed JC (2001) Molecular chaperone targeting and regulation by bag family proteins. Nat Cell Biol 3:E237–E241
27. Briknarova K, Takayama S, Homma S, Baker K, Cabezas E, Hoyt DW, Li Z, Satterthwait AC, Ely KR (2002) Bag4/sodd protein contains a short bag domain. J Biol Chem 277:31172–31178
28. Takayama S, Sato T, Krajewski S, Kochel K, Irie S, Millan JA, Reed JC (1995) Cloning and functional analysis of bag-1: a novel bcl-2-binding protein with anti-cell death activity. Cell 80:279–284
29. Jiang Y, Woronicz JD, Liu W, Goeddel DV (1999) Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science 283:543–546
30. Wang HG, Reed JC (1998) Bcl-2, raf-1 and mitochondrial regulation of apoptosis. BioFactors 8:13–16
31. Ingham RJ, Colwill K, Howard C, Dettwiler S, Lim CS, Yu J, Hersi K, Raaijmakers J, Gish G, Mbamalu G, Taylor L, Yeung B, Vassilovski G, Amin M, Chen F, Matskova L, Winberg G, Ernberg I, Linding R, O'Donnell P, Starostine A, Keller W, Metalnikov P, Stark C, Pawson T (2005) Ww domains provide a platform for the assembly of multiprotein networks. Mol Cell Biol 25:7092–7106
32. Miki K, Eddy EM (2002) Tumor necrosis factor receptor 1 is an atpase regulated by silencer of death domain. Mol Cell Biol 22:2536–2543
33. Thress K, Henzel W, Shillinglaw W, Kornbluth S (1998) Scythe: a novel reaper-binding apoptotic regulator. EMBO J 17:6135–6143
34. Thress K, Song J, Morimoto RI, Kornbluth S (2001) Reversible inhibition of hsp70 chaperone function by scythe and reaper. EMBO J 20:1033–1041

35. Andre B, Springael JY (1994) Wwp, a new amino acid motif present in single or multiple copies in various proteins including dystrophin and the sh3-binding yes-associated protein yap65. Biochem Biophys Res Commun 205:1201–1205
36. Doong H, Price J, Kim YS, Gasbarre C, Probst J, Liotta LA, Blanchette J, Rizzo K, Kohn E (2000) Cair-1/bag-3 forms an egf-regulated ternary complex with phospholipase c-gamma and hsp70/hsc70. Oncogene 19:4385–4395
37. Li SS (2005) Specificity and versatility of SH3 and other proline-recognition domains: structural basis and implications for cellular signal transduction. Biochem J 390:641–653
38. Hishiya A, Salman MN, Carra S, Kampinga HH, Takayama S (2011) Bag3 directly interacts with mutated alphab-crystallin to suppress its aggregation and toxicity. PLoS ONE 6:e16828
39. Carra S, Sivilotti M, Chavez Zobel AT, Lambert H, Landry J (2005) Hspb8, a small heat shock protein mutated in human neuromuscular disorders, has in vivo chaperone activity in cultured cells. Hum Mol Genet 14:1659–1669
40. Carra S, Seguin SJ, Landry J (2008) Hspb8 and bag3: a new chaperone complex targeting misfolded proteins to macroautophagy. Autophagy 4:237–239
41. Chang JS, Kim SK, Kwon TK, Bae SS, Min DS, Lee YH, Kim SO, Seo JK, Choi JH, Suh PG (2005) Pleckstrin homology domains of phospholipase c-gamma1 directly interact with beta-tubulin for activation of phospholipase c-gamma1 and reciprocal modulation of beta-tubulin function in microtubule assembly. J Biol Chem 280:6897–6905
42. Pagliuca MG, Lerose R, Cigliano S, Leone A (2003) Regulation by heavy metals and temperature of the human bag-3 gene, a modulator of hsp70 activity. FEBS Lett 541:11–15
43. Liao Q, Ozawa F, Friess H, Zimmermann A, Takayama S, Reed JC, Kleeff J, Buchler MW (2001) The anti-apoptotic protein bag-3 is overexpressed in pancreatic cancer and induced by heat stress in pancreatic cancer cell lines. FEBS Lett 503:151–157
44. Bonelli P, Petrella A, Rosati A, Romano MF, Lerose R, Pagliuca MG, Amelio T, Festa M, Martire G, Venuta S, Turco MC, Leone A (2004) Bag3 protein regulates stress-induced apoptosis in normal and neoplastic leukocytes. Leukemia 18:358–360
45. Wang HQ, Liu HM, Zhang HY, Guan Y, Du ZX (2008) Transcriptional upregulation of bag3 upon proteasome inhibition. Biochem Biophys Res Commun 365:381–385
46. Li C, Li S, Kong DH, Meng X, Zong ZH, Liu BQ, Guan Y, Du ZX, Wang HQ (2013) Bag3 is upregulated by c-jun and stabilizes jund. Biochim Biophys Acta 1833:3346–3354
47. Chen L, Wu W, Dentchev T, Zeng Y, Wang J, Tsui I, Tobias JW, Bennett J, Baldwin D, Dunaief JL (2004) Light damage induced changes in mouse retinal gene expression. Exp Eye Res 79:239–247
48. Lee MY, Kim SY, Choi JS, Choi YS, Jeon MH, Lee JH, Kim IK (2002) Induction of bis, a bcl-2-binding protein, in reactive astrocytes of the rat hippocampus following kainic acid-induced seizure. Exp Mol Med 34:167–171
49. Friedrich B, Janessa A, Schmieder R, Risler T, Alexander D (2008) Acute effects of haemodialysis on pro-/anti- apoptotic genes in peripheral blood leukocytes. Cell Physiol Biochem 22:423–430
50. Jung SE, Kim YK, Youn DY, Lim MH, Ko JH, Ahn YS, Lee JH (2010) Down-modulation of bis sensitizes cell death in c6 glioma cells induced by oxygen–glucose deprivation. Brain Res 1349:1–10
51. Yan H, Frost P, Shi Y, Hoang B, Sharma S, Fisher M, Gera J, Lichtenstein A (2006) Mechanism by which mammalian target of rapamycin inhibitors sensitize multiple myeloma cells to dexamethasone-induced apoptosis. Cancer Res 66:2305–2313
52. Rosati A, Basile A, Falco A, d'Avenia M, Festa M, Graziano V, De Laurenzi V, Arra C, Pascale M, Turco MC (2012) Role of bag3 protein in leukemia cell survival and response to therapy. Biochim Biophys Acta 1826:365–369
53. Romano MF, Festa M, Pagliuca G, Lerose R, Bisogni R, Chiurazzi F, Storti G, Volpe S, Venuta S, Turco MC, Leone A (2003) Bag3 protein controls b-chronic lymphocytic leukaemia cell apoptosis. Cell Death Differ 10:383–385
54. Chiappetta G, Ammirante M, Basile A, Rosati A, Festa M, Monaco M, Vuttariello E, Pasquinelli R, Arra C, Zerilli M, Todaro M, Stassi G, Pezzullo L, Gentilella A, Tosco A, Pascale M, Marzullo L, Belisario MA, Turco MC, Leone A (2007) The antiapoptotic protein bag3 is expressed in thyroid carcinomas and modulates apoptosis mediated by tumor necrosis factor-related apoptosis-inducing ligand. J Clin Endocrinol Metab 92:1159–1163
55. Franco R, Scognamiglio G, Salerno V, Sebastiani A, Cennamo G, Ascierto PA, Botti G, Turco MC, Rosati A (2012) Expression of the anti-apoptotic protein bag3 in human melanomas. J Investig Dermatol 132:252–254
56. Zhang Y, Wang JH, Lu Q, Wang YJ (2012) Bag3 promotes resistance to apoptosis through bcl-2 family members in non-small cell lung cancer. Oncol Rep 27:109–113
57. Xiao H, Cheng S, Tong R, Lv Z, Ding C, Du C, Xie H, Zhou L, Wu J, Zheng S (2014) Bag3 regulates epithelial–mesenchymal transition and angiogenesis in human hepatocellular carcinoma. Lab Investig 94:252–261
58. Rosati A, Bersani S, Tavano F, Dalla Pozza E, De Marco M, Palmieri M, De Laurenzi V, Franco R, Scognamiglio G, Palaia R, Fontana A, di Sebastiano P, Donadelli M, Dando I, Medema JP, Dijk F, Welling L, di Mola FF, Pezzilli R, Turco MC, Scarpa A (2012) Expression of the antiapoptotic protein bag3 is a feature of pancreatic adenocarcinoma and its overexpression is associated with poorer survival. Am J Pathol 181:1524–1529
59. Chiappetta G, Basile A, Barbieri A, Falco A, Rosati A, Festa M, Pasquinelli R, Califano D, Palma G, Costanzo R, Barcaroli D, Capunzo M, Franco R, Rocco G, Pascale M, Turco MC, De Laurenzi V, Arra C (2014) The anti-apoptotic bag3 protein is expressed in lung carcinomas and regulates small cell lung carcinoma (scle) tumor growth. Oncotarget 5:6846–6853
60. Festa M, Del Valle L, Khalili K, Franco R, Scognamiglio G, Graziano V, De Laurenzi V, Turco MC, Rosati A (2011) Bag3 protein is overexpressed in human glioblastoma and is a potential target for therapy. Am J Pathol 178:2504–2512
61. Iwasaki M, Homma S, Hishiya A, Dolezal SJ, Reed JC, Takayama S (2007) Bag3 regulates motility and adhesion of epithelial cancer cells. Cancer Res 67:10252–10259
62. Gentilella A, Khalili K (2009) Autoregulation of co-chaperone bag3 gene transcription. J Cell Biochem 108:1117–1124
63. Franceschelli S, Rosati A, Lerose R, De Nicola S, Turco MC, Pascale M (2008) Bag3 gene expression is regulated by heat shock factor 1. J Cell Physiol 215:575–577
64. Ulbricht A, Eppler FJ, Tapia VE, van der Ven PF, Hampe N, Hersch N, Vakeel P, Stadel D, Haas A, Saftig P, Behrends C, Furst DO, Volkmer R, Hoffmann B, Kolanus W, Hohfeld J (2013) Cellular mechanotransduction relies on tension-induced and chaperone-assisted autophagy. Curr Biol 23:430–435
65. Yang L, Han Y, Suarez Saiz F, Minden MD (2007) A tumor suppressor and oncogene: the wt1 story. Leukemia 21:868–876
66. Cesaro E, Montano G, Rosati A, Crescitelli R, Izzo P, Turco MC, Costanzo P (2010) Wt1 protein is a transcriptional activator of the antiapoptotic bag3 gene. Leukemia 24:1204–1206
67. Gentilella A, Passiatore G, Deshmane S, Turco MC, Khalili K (2008) Activation of bag3 by egr-1 in response to fgf-2 in neuroblastoma cells. Oncogene 27:5011–5018
68. Ben Aicha S, Lessard J, Pelletier M, Fournier A, Calvo E, Labric C (2007) Transcriptional profiling of genes that are regulated by the endoplasmic reticulum-bound transcription factor nibzip/creb3l4 in prostate cells. Physiol Genomics 31:295–305

69. Steinberg SF (2004) Distinctive activation mechanisms and functions for protein kinase cdelta. Biochem J 384:449–459
70. Li N, Du ZX, Zong ZH, Liu BQ, Li C, Zhang Q, Wang HQ (2013) Pkcdelta-mediated phosphorylation of bag3 at ser187 site induces epithelial-mesenchymal transition and enhances invasiveness in thyroid cancer fro cells. Oncogene 32:4539–4548
71. Bruno AP, De Simone FI, Iorio V, De Marco M, Khalili K, Sariyer IK, Capunzo M, Nori SL, Rosati A (2014) Hiv-1 tat protein induces glial cell autophagy through enhancement of bag3 protein levels. Cell Cycle 13:3640–3644
72. Du ZX, Meng X, Zhang HY, Guan Y, Wang HQ (2008) Caspase-dependent cleavage of bag3 in proteasome inhibitors-induced apoptosis in thyroid cancer cells. Biochem Biophys Res Commun 369:894–898
73. Liu BQ, Du ZX, Zong ZH, Li C, Li N, Zhang Q, Kong DH, Wang HQ (2013) Bag3-dependent noncanonical autophagy induced by proteasome inhibition in hepg2 cells. Autophagy 9:905–916
74. Wang HQ, Meng X, Liu BQ, Li C, Gao YY, Niu XF, Li N, Guan Y, Du ZX (2012) Involvement of jnk and nf-kappab pathways in lipopolysaccharide (lps)-induced bag3 expression in human monocytic cells. Exp Cell Res 318:16–24
75. Zhong W, Zhu H, Sheng F, Tian Y, Zhou J, Chen Y, Li S, Lin J (2014) Activation of the mapk11/12/13/14 (p38 mapk) pathway regulates the transcription of autophagy genes in response to oxidative stress induced by a novel copper complex in hela cells. Autophagy 10:1285–1300
76. Nandi D, Tahiliani P, Kumar A, Chandu D (2006) The ubiquitin-proteasome system. J Biosci 31:137–155
77. Merabova N, Sariyer IK, Saribas AS, Knezevic T, Gordon J, Turco MC, Rosati A, Weaver M, Landry J, Khalili K (2015) Ww domain of bag3 is required for the induction of autophagy in glioma cells. J Cell Physiol 230:831–841
78. Gamerdinger M, Carra S, Behl C (2011) Emerging roles of molecular chaperones and co-chaperones in selective autophagy: focus on bag proteins. J Mol Med 89:1175–1182
79. Hetz C, Thielen P, Matus S, Nassif M, Court F, Kiffin R, Martinez G, Cuervo AM, Brown RH, Glimcher LH (2009) Xbp-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. Genes Dev 23:2294–2306
80. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ, Rubinsztein DC (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of huntington disease. Nat Genet 36:585–595
81. Sarkar S, Ravikumar B, Floto RA, Rubinsztein DC (2009) Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. Cell Death Differ 16:46–56
82. Seidel K, Vinet J, Dunnen WF, Brunt ER, Meister M, Boncoraglio A, Zijlstra MP, Boddeke HW, Rub U, Kampinga HH, Carra S (2012) The hspb8-bag3 chaperone complex is upregulated in astrocytes in the human brain affected by protein aggregation diseases. Neuropathol Appl Neurobiol 38:39–53
83. Lei Z, Brizzee C, Johnson GV (2015) Bag3 facilitates the clearance of endogenous tau in primary neurons. Neurobiol Aging 36:241–248
84. Doong H, Vrailas A, Kohn EC (2002) What's in the 'bag'?—a functional domain analysis of the bag-family proteins. Cancer Lett 188:25–32
85. Carra S, Seguin SJ, Lambert H, Landry J (2008) Hspb8 chaperone activity toward poly(q)-containing proteins depends on its association with bag3, a stimulator of macroautophagy. J Biol Chem 283:1437–1444
86. Fuchs MPD, Seguin SJ, Lambert H, Carra S, Charette SJ, Landry J (2009) Identification of the key structural motifs involved in hspb8/hspb6-bag3 interaction. Biochem J 425:245–255
87. Crippa V, Sau D, Rusmini P, Boncoraglio A, Onesto E, Bolzoni E, Galbiati M, Fontana E, Marino M, Carra S, Bendotti C, De Biasi S, Poletti A (2010) The small heat shock protein b8 (hspb8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (als). Hum Mol Genet 19:3440–3456
88. Gamerdinger M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C (2009) Protein quality control during aging involves recruitment of the macroautophagy pathway by bag3. EMBO J 28:889–901
89. Gamerdinger M, Kaya AM, Wolfrum U, Clement AM, Behl C (2011) Bag3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins. EMBO Rep 12:149–156
90. Bjorkoy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H, Johansen T (2005) P62/sqstm1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol 171:603–614
91. Roberts AJ, Kon T, Knight PJ, Sutoh K, Burgess SA (2013) Functions and mechanics of dynein motor proteins. Nat Rev Mol Cell Biol 14:713–726
92. Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 10:524–530
93. Garcia-Mata R, Bebok Z, Sorscher EJ, Sztul ES (1999) Characterization and dynamics of aggresome formation by a cytosolic gfp-chimera. J Cell Biol 146:1239–1254
94. Johnston JA, Ward CL, Kopito RR (1998) Aggresomes: a cellular response to misfolded proteins. J Cell Biol 143:1883–1898
95. Martin SJ, Green DR (1995) Protease activation during apoptosis: death by a thousand cuts? Cell 82:349–352
96. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J (1996) Human ice/ced-3 protease nomenclature. Cell 87:171
97. Thornberry NA, Lazebnik Y (1998) Caspases: enemies within. Science 281:1312–1316
98. Adams JM, Cory S (1998) The bcl-2 protein family: arbiters of cell survival. Science 281:1322–1326
99. Chinnaiyan AM, Orth K, O'Rourke K, Duan H, Poirier GG, Dixit VM (1996) Molecular ordering of the cell death pathway. Bcl-2 and bcl-xl function upstream of the ced-3-like apoptotic proteases. J Biol Chem 271:4573–4576
100. Boulakia CA, Chen G, Ng FW, Teodoro JG, Branton PE, Nicholson DW, Poirier GG, Shore GC (1996) Bcl-2 and adenovirus e1b 19 kDa protein prevent e1a-induced processing of cpp32 and cleavage of poly(adp-ribose) polymerase. Oncogene 12:529–535
101. Shimizu S, Eguchi Y, Kamiike W, Matsuda H, Tsujimoto Y (1996) Bcl-2 expression prevents activation of the ice protease cascade. Oncogene 12:2251–2257
102. Armstrong RC, Aja T, Xiang J, Gaur S, Krebs JF, Hoang K, Bai X, Korsmeyer SJ, Karanewsky DS, Fritz LC, Tomaselli KJ (1996) Fas-induced activation of the cell death-related protease cpp32 is inhibited by bcl-2 and by ice family protease inhibitors. J Biol Chem 271:16850–16855
103. Chinnaiyan AM, O'Rourke K, Lane BR, Dixit VM (1997) Interaction of ced-4 with ced-3 and ced-9: a molecular framework for cell death. Science 275:1122–1126
104. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD (1997) The release of cytochrome c from mitochondria: a primary site for bcl-2 regulation of apoptosis. Science 275:1132–1136
105. Ammirante M, Rosati A, Arra C, Basile A, Falco A, Festa M, Pascale M, d'Avenia M, Marzullo L, Belisario MA, De Marco M, Barbieri A, Giudice A, Chiappetta G, Vuttariello E, Monaco M, Bonelli P, Salvatore G, Di Benedetto M, Deshmane SL, Khalili K, Turco MC, Leone A (2010) Ikk{gamma} protein is a target of bag3 regulatory activity in human tumor growth. Proc Natl Acad Sci USA 107:7497–7502

106. Nivon M, Abou-Samra M, Richet E, Guyot B, Arrigo AP, Kretz-Remy C (2012) Nf-kappab regulates protein quality control after heat stress through modulation of the bag3-hspb8 complex. J Cell Sci 125:1141–1151
107. Falco A, Festa M, Basile A, Rosati A, Pascale M, Florenzano F, Nori SL, Nicolin V, Di Benedetto M, Vecchione ML, Arra C, Barbieri A, De Laurenzi V, Turco MC (2012) Bag3 controls angiogenesis through regulation of erk phosphorylation. Oncogene 31:5153–5161
108. Meng X, Kong DH, Li N, Zong ZH, Liu BQ, Du ZX, Guan Y, Cao L, Wang HQ (2014) Knockdown of bag3 induces epithelial-mesenchymal transition in thyroid cancer cells through zeb1 activation. Cell Death Dis 5:e1092
109. Colvin TA, Gabai VL, Gong J, Calderwood SK, Li H, Gummuluru S, Matchuk ON, Smirnova SG, Orlova NV, Zamulaeva IA, Garcia-Marcos M, Li X, Young ZT, Rauch JN, Gestwicki JE, Takayama S, Sherman MY (2014) Hsp70-bag3 interactions regulate cancer-related signaling networks. Cancer Res 74:4731–4740
110. Sugio A, Iwasaki M, Habata S, Mariya T, Suzuki M, Osogami H, Tamate M, Tanaka R, Saito T (2014) Bag3 upregulates mcl-1 through downregulation of mir-29b to induce anticancer drug resistance in ovarian cancer. Gynecol Oncol 134:615–623
111. Selcen D, Engel AG (2011) Myofibrillar myopathies. Handb Clin Neurol 101:143–154
112. Pappas CT, Krieg PA, Gregorio CC (2010) Nebulin regulates actin filament lengths by a stabilization mechanism. J Cell Biol 189:859–870
113. Pappas CT, Bhattacharya N, Cooper JA, Gregorio CC (2008) Nebulin interacts with capz and regulates thin filament architecture within the z-disc. Mol Biol Cell 19:1837–1847
114. Schafer DA, Hug C, Cooper JA (1995) Inhibition of capz during myofibrillogenesis alters assembly of actin filaments. J Cell Biol 128:61–70
115. Knoll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, Hayashi T, Shiga N, Yasukawa H, Schaper W, McKenna W, Yokoyama M, Schork NJ, Omens JH, McCulloch AD, Kimura A, Gregorio CC, Poller W, Schaper J, Schultheiss HP, Chien KR (2002) The cardiac mechanical stretch sensor machinery involves a z disc complex that is defective in a subset of human dilated cardiomyopathy. Cell 111:943–955
116. Engel AG (1999) Myofibrillar myopathy. Ann Neurol 46:681–683
117. van der Ven PF, Schaart G, Croes HJ, Jap PH, Ginsel LA, Ramaekers FC (1993) Titin aggregates associated with intermediate filaments align along stress fiber-like structures during human skeletal muscle cell differentiation. J Cell Sci 106(Pt 3):749–759
118. McCollum AK, Casagrande G, Koch EC (2010) Caught in the middle: the role of Bag3 in disease. Biochem J 425:e1–e3
119. Tilley DG, Zhu W, Myers VD, Barr LA, Gao E, Li X, Song J, Carter RL, Makarewich CA, Yu D, Troupes CD, Grisanti LA, Coleman RC, Koch WJ, Houser SR, Cheung JY, Feldman AM (2014) T1 β-adrenergic receptor-mediated cardiac contractility is inhibited via vasopressin type 1A-receptor-dependent signaling. Circulation 130(20):1800–1811

EXHIBIT "D"

US 20170016066A1

(19) **United States**

(12) **Patent Application Publication**

FELDMAN et al.

(10) **Pub. No.: US 2017/0016066 A1**

(43) **Pub. Date: Jan. 19, 2017**

(54) **BAG3 AS A TARGET FOR THERAPY OF HEART FAILURE**

(71) Applicant: **TEMPLE UNIVERSITY OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION**, Philadelphia, PA (US)

(72) Inventors: **Arthur M. FELDMAN**, Wynnewood, PA (US); **Douglas G. TILLEY**, Bryn Mawr, PA (US); **Weizhong ZHU**, Cockeysville, MD (US); **Kamel KHALILI**, Bala Cynwyd, PA (US); **Walter J. KOCH**, Broomall, PA (US)

(73) Assignee: **Temple University of the Commonwealth System of Higher Education**, Philadelphia, PA (US)

(21) Appl. No.: **15/115,807**

(22) PCT Filed: **Jan. 30, 2015**

(86) PCT No.: **PCT/US15/13926**

§ 371 (c)(1),
(2) Date: **Aug. 1, 2016**

Related U.S. Application Data

(60) Provisional application No. 61/934,483, filed on Jan. 31, 2014.

Publication Classification

(51) **Int. Cl.**
C12Q 1/68 (2006.01)
G01N 33/68 (2006.01)

(52) **U.S. Cl.**
CPC *C12Q 1/6883* (2013.01); *G01N 33/6887* (2013.01); *C12Q 2600/156* (2013.01); *G01N 2800/325* (2013.01); *C12Q 2600/106* (2013.01)

(57) **ABSTRACT**

Compositions are directed to BCL2-associated athanogene 3 (BAG3) molecules and agents which modulate expression of BAG3 molecules. Pharmaceutical composition for administration to patients, for example, patients with heart failure, comprise one or more BAG3 molecules or agents which modulate expression of BAG3. Methods of treatment and identifying candidate therapeutic agents are also provided.

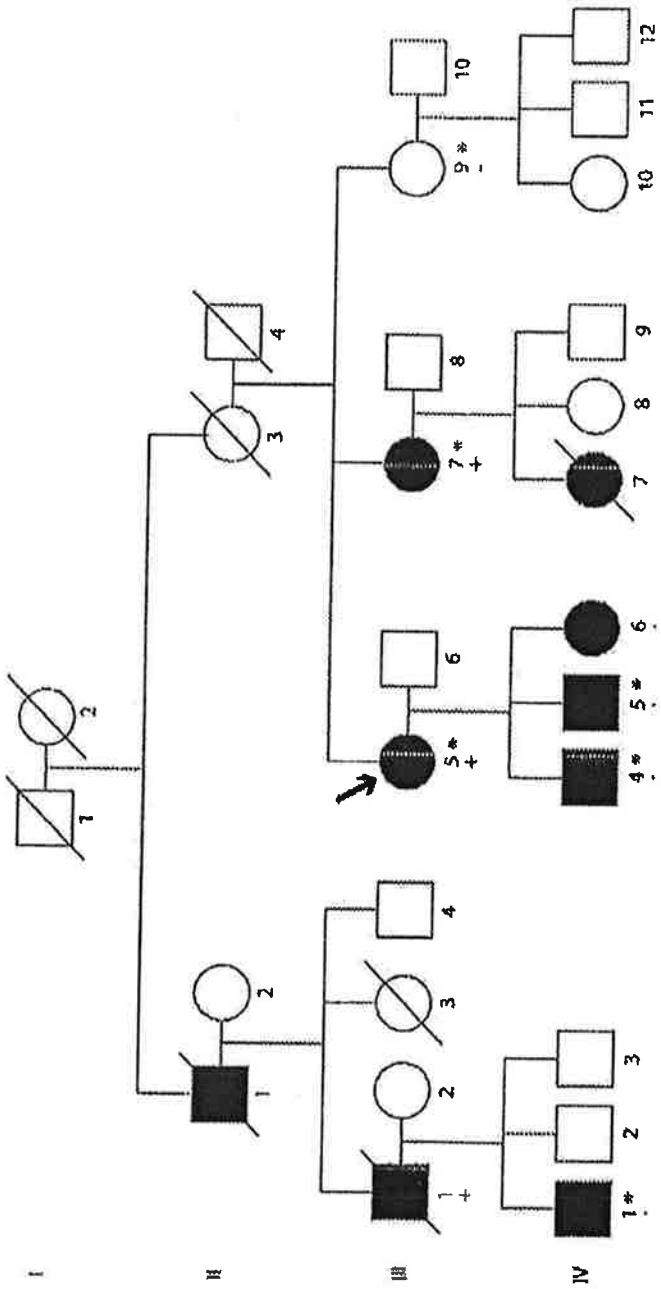


FIG. 1

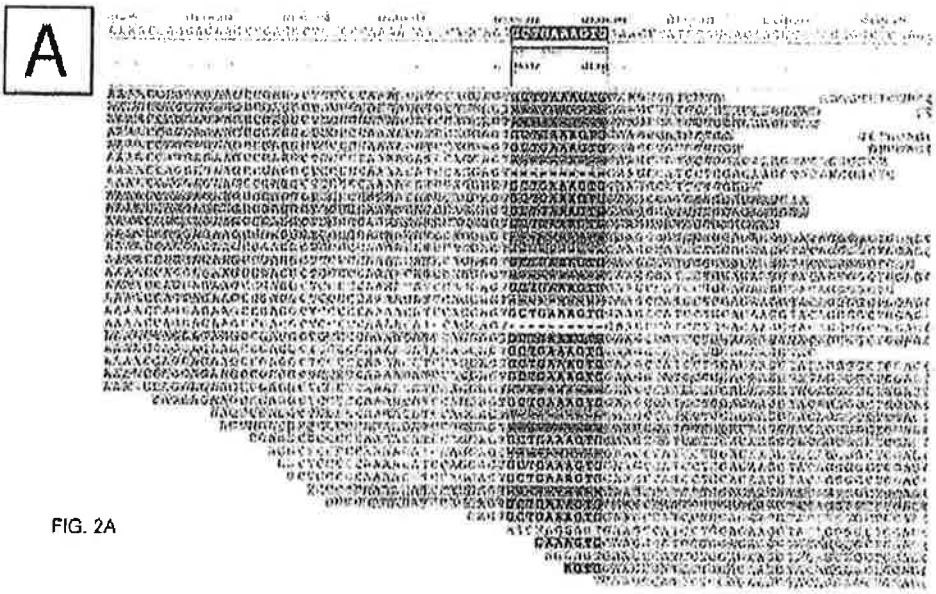


FIG. 2A

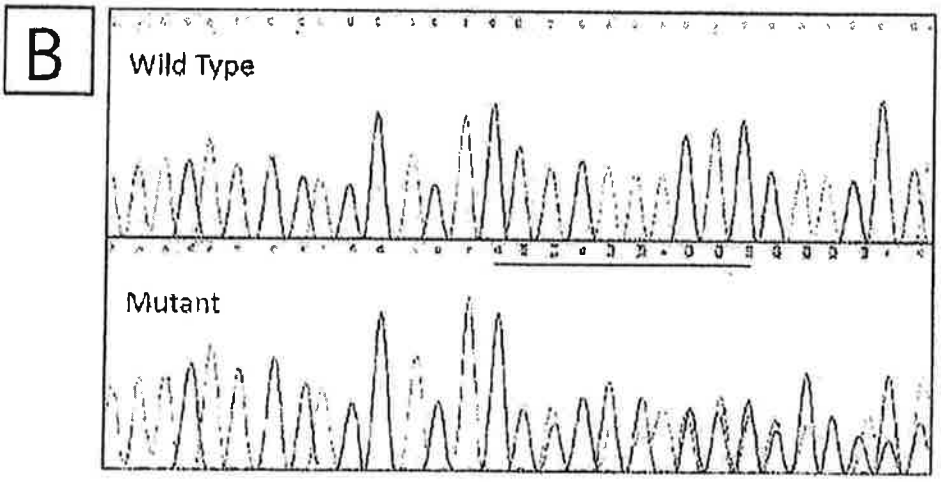


FIG. 2B

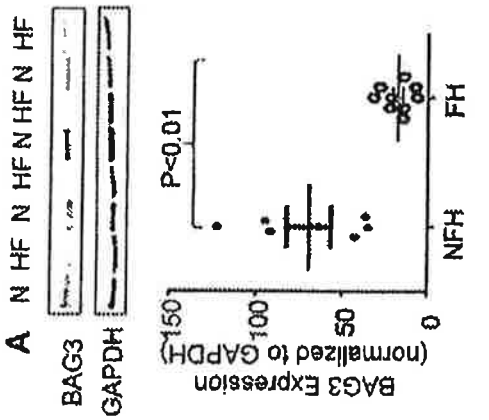


FIG. 3A

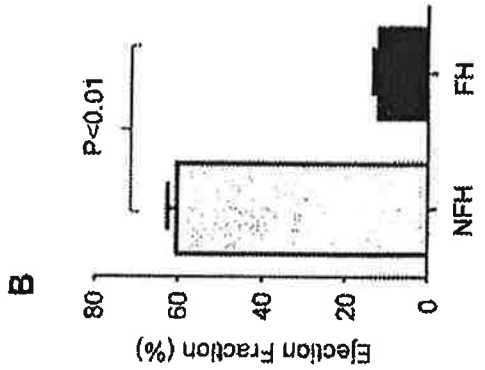


FIG. 3B

Figure 3A, 3B. Low level expression of BAG3 in patients with heart failure

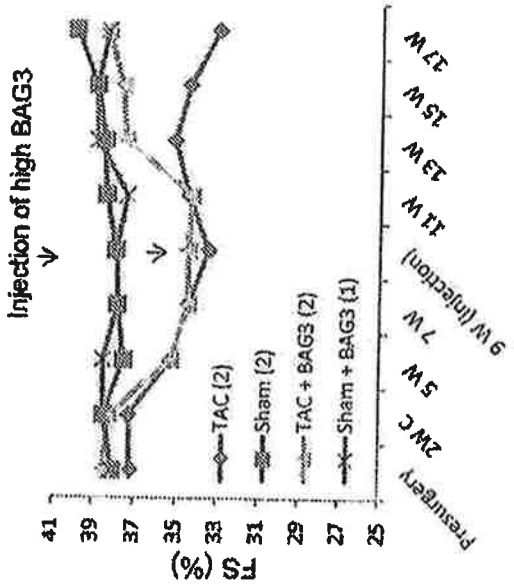

Injection of high BAG3
TAC (2)
Sham (2)
TAC + BAG3 (2)
Sham + BAG3 (1)
FS (%)
41
39
37
35
33
31
29
27
25
Pre
2W C
5W
7W
9W (Injection)
11W
13W
15W
17W

FIG. 4

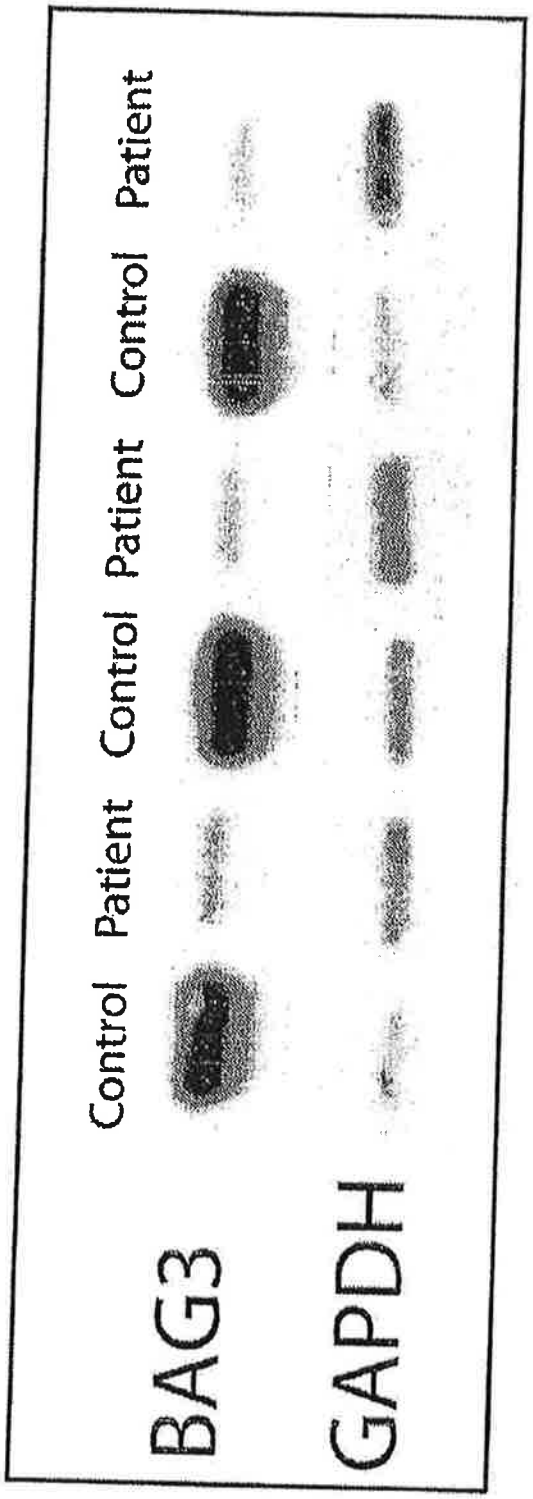

BAG3
GAPDH
Control
Patient
Control
Patient
Control
Patient

FIG. 5

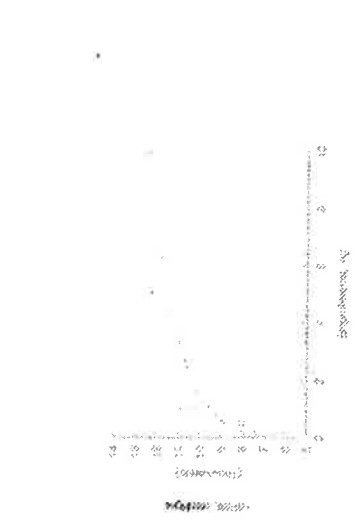


FIG. 6B

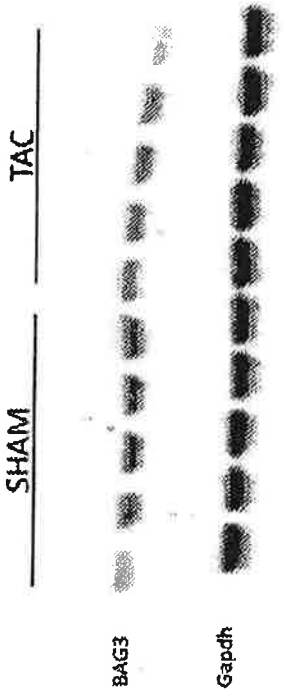


FIG. 6D

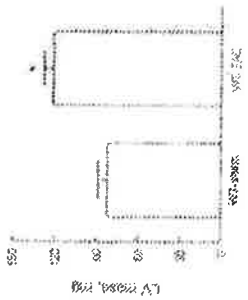


FIG. 6A

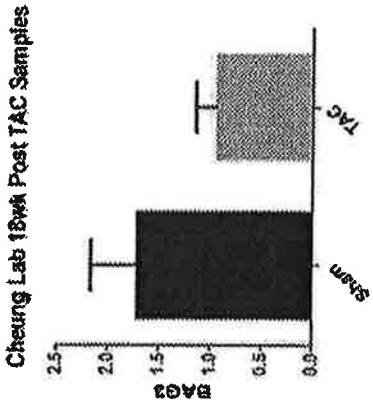


FIG. 6C

18 Week TAC Mice

Unpaired T-test P=0.0073

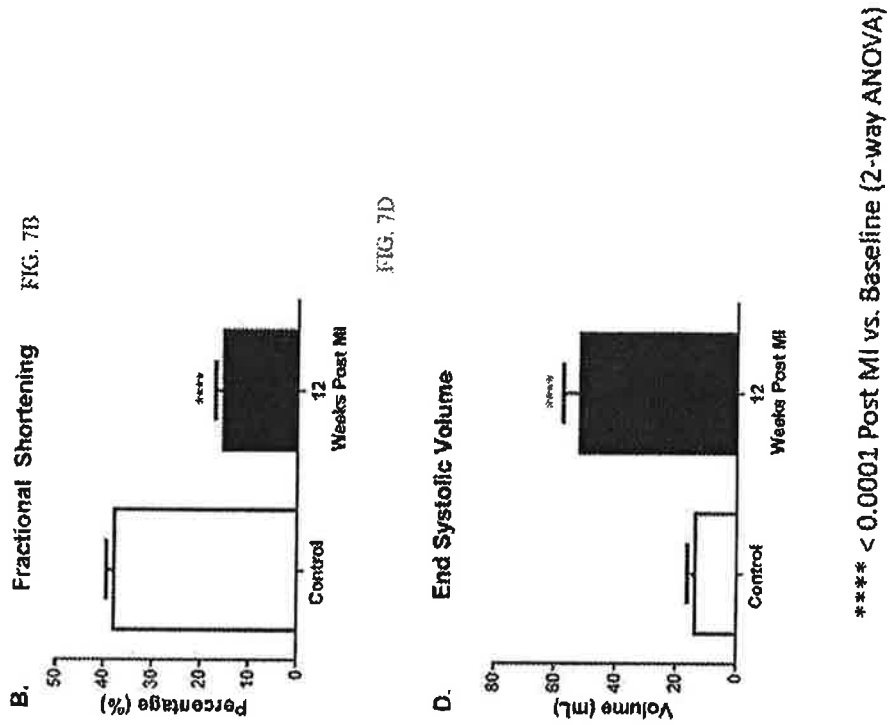


FIG. 7A

FIG. 7B

FIG. 7C

FIG. 7D

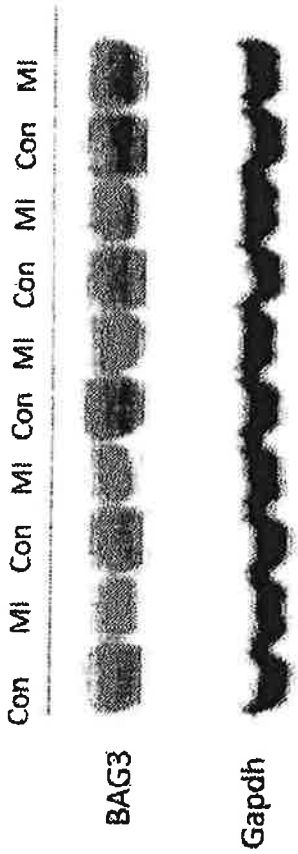

Con
MI
Con
MI
Con
MI
Con
MI
Con
MI
BAG3
Gapdh

FIG. 7E

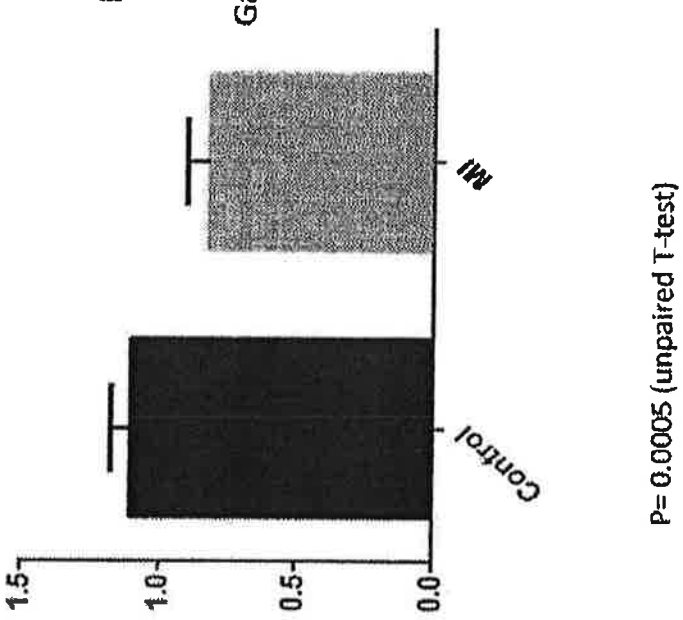

1.5
1.0
0.5
0.0
Control
MI
P= 0.0005 (unpaired T-test)

FIG. 7F

FIG. 8

Exemplary Human BAG3 Polypeptide Sequence
(Genbank NP_004272.2; Public GI:14043024

```
  1 maaathspmm qvasgngdrd plppgweiki dpqtgwpffv dhnrrtttwn dprvpaegpk
 61 etpssangps regsrlppar eghpvypqlr pgyipipvlh egaenrqvhp fhvypqpgmq
121 rfrtaaaaaa pqrsqsplrq mpettqpdkq cgqvaaaaaa qppashgper gqspaaasdc
181 ssssaaslps sqrsslgshq lprgylsipv rheqnvtrpa aqpsfhqaqk thypaqqgey
241 qthqpvyhki qgddweprpl raaspfrssv qgaasregsp arsstplhsp spirvhtvvd
301 rpqqpmthre tapvsqpenk peskpgpvgp elppghipiq virkevdskp vsqkppppae
361 kvevkvppap vpcpppspqp aavpaepksv ateeraapst apaaatppkp geaeappkhp
421 gvlkveaile kvqgleqavd nfeqkkndkk yimieeyltk ellaldsvdp egraadvrqar
481 rdqvrkvqti lekleqkaid vpgqvqvyel qpsnleadqp lqaimemgav aadkgkknag
541 naedphtetq qpeataaats npssmtdtpg npaap (SEQ ID NO.1)
```

BAG3 AS A TARGET FOR THERAPY OF HEART FAILURE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of the filing date of U.S. Provisional Application No. 61/934,483, which was filed on Jan. 31, 2014. For the purpose of any U.S. application that may claim the benefit of U.S. Provisional Application No. 61/934,483, the contents of that earlier filed application are hereby incorporated by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with U.S. government support under grant number P01 HL091799 awarded by the National Institutes of Health. The U.S. government may have certain rights in the invention.

FIELD OF THE INVENTION

[0003] Embodiments of the invention are directed to compositions for the treatment of cardiac diseases or disorders, such as heart failure, cardiovascular diseases or disorders, or skeletal muscle diseases associated with Bcl-2 associated anthanogene-3 (BAG3) expression, and methods of treatment. Assays for the identification of novel therapeutic agents are also provided.

BACKGROUND

[0004] Heart failure (HF), secondary to systolic dysfunction and cardiac dilatation affects over 5 million individuals in the U.S. and is an important cause of both morbidity and mortality. Approximately 30% of these patients have non-ischemic disease or idiopathic dilated cardiomyopathy (IDC). Although in the majority of patients with IDC the causative factors have remained undefined, emerging evidence suggests that up to 35% of individuals with IDC have an affected first degree relative (Jefferies J L T J. *Lancet.* 2010;375:752-762) and IDC can be associated with genetic abnormalities in 20-35% of individuals—leading to the use of the nomenclature familial dilated cardiomyopathy (FDC) (Judge D P et al, *Journal of Cardiovascular Translational Research.* 2008; 1:144-154; Hershberger R E et al., *Circulation. Cardiovascular Genetics.* 2010; 3:155-161). Indeed, mutations in more than 30 genes have been identified as causative factors (Hershberger R E, et al., *Circulation. Heart Failure.* 2009; 2:253-261) and the most common pattern of inheritance is autosomal dominant with reduced penetrance and variable expressivity (Morales A, Hershberger R E. *Current Cardiology Reports.* 2013; 15:375).

[0005] Mutations causing FDC are found in genes encoding a wide spectrum of proteins[6]; however, a large number of the mutations that cause FDC occur in genes that encode sarcomere proteins or the complex network of proteins in the Z-disc(Chang A N, Potter J D. *Heart Failure Reviews.* 2005; 10:225-235; Selcen D. Myofibrillar myopathies. *Neuromuscular disorders: NMD.* 2011; 21:161-171).

SUMMARY

[0006] Embodiments of the invention are directed to compositions for modulating the expression of Bcl-2 associated anthanogene-3 (BAG3) molecules, methods for identifying agents for treatment of cardiac diseases or disorders. In particular, these agents comprise expression vectors encoding Bcl-2 associated anthanogene-3 (BAG3) molecules, Bcl-2 associated anthanogene-3 (BAG3) nucleic acid sequences, Bcl-2 associated anthanogene-3 (BAG3) peptides or any other agent which modulates BAG3 expression. Such agents are identified by methods embodied herein. Conditions that are treated include, for example, heart failure, cardiomyopathy and the like. In some embodiments, the target tissues are cardiac tissues, such as for example, heart muscle.

[0007] Briefly, the results obtained herein have identified a rare and novel variant in a family with familial dilated cardiomyopathy. General embodiments of the invention are directed to treatment of patients identified as having variants of BAG3 molecules.

[0008] Patients with idiopathic dilated cardiomyopathy who did not have a mutation in the BAG3 gene were found to have half the normal level of BAG3, the same decrease that was found in the heart of the patient with the familial disease and the BAG3 mutation. Other general embodiments of the invention to treatment of patients with agents which modulate expression of BAG3 molecules, preferably resulting in overexpression of normal BAG3 molecules.

[0009] The results also showed that mice with heart failure due to aortic banding (a commonly used model for heart failure studies) had substantially less BAG3 than normal controls—and a reduction in BAG3 that mirrored that seen in humans.

[0010] Results also showed that when an AAV vector (AAV9) was administered in vivo, to over-express BAG3 in the heart, robust over-expression was observed. In other general embodiments, an agent comprises a cardiotropic vector expressing a BAG3 molecule. In some embodiments, the vector is an AAV9 vector.

[0011] It was also found that when the BAG3 protein was over-expressed in the hearts of mice with heart failure secondary to aortic banding (and low levels of BAG3) by using the AAV9 vector, normal left ventricular performance was reconstituted. These results provide evidence that BAG3 levels are decreased in the failing mouse heart and the AAV9 vector over-expressed BAG3 in the desired target in the heart resulting in the change in function comparable to a normal function.

[0012] Other aspects are described infra.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 is a schematic representation showing the BAG3-Associated Dilated Cardiomyopathy Pedigree. Males are represented by squares. Circles indicate females. Open symbols represent unaffected individuals and black symbols represent affected individuals. The presence or absence of the 10-nucleotide deletion in BAG3 is indicated by either a (+) or a (−) respectively. An arrow denotes the proband. An asterisk is used to denote individuals whose DNA was used for whole exome sequencing. A diagonal line is used to denote individuals who are deceased.

[0014] FIG. 2A is a schematic representation showing the sequencing alignment for BAG3 10-nucleotide deletion. FIG. 2B is a schematic representation showing the representative Sanger sequencing of the deletion in the BAG3 gene in an affected individual.

[0015] FIGS. 3A and 3B show the low level expression of BAG3 in patients with heart failure. A representative Western blot of BAG3 and GAPDH levels in non-failing (NF) and failing (F) human heart is shown. The graph shows the quantification of BAG3 protein levels in non-failing and failing human heart. Values are normalized to the level of GAPDH in order to account for variations in protein loading. Horizontal lines represent mean and standard error of the mean. Statistical analysis was performed using unpaired t-test with Welch's correction for unequal variance.

[0016] FIG. 4 shows the measurement of ejection fraction in wild type sham operated mice, wild type mice that have been injected with the AAV9-BAG3 construct, mice that have undergone aortic banding (and developed heart failure) and mice that have been banded and in heart failure but were injected with AAV9-BAG3. As can be seen, the BAG3 injection normalized LV function temporally related to the expression of the BAG3 protein (approximately 5 to 6 weeks after injection).

[0017] FIG. 5 is a Western Blot showing BAG3 levels in patients. Three lanes of "control" and three lanes of "patient" are shown. This is patient IV-1—who is an affected. The "control" lanes are from a non-failing human heart—i.e. Normal human heart—that was obtained at the time of tissue harvest but could not be used for transplant because of size or tissue type incompatibility with available recipients. All of the lanes labeled "patient" were from the same patient—IV-1. These were obtained from pieces of his heart that were explanted at the time he underwent a heart transplant. The results show that the decrease in BAG3 levels are comparable to the decrease that was seen in the patients with non-familial heart failure.

[0018] FIGS. 6A-6D show BAG3 levels in failing murine hearts. FIG. 6A is a graph depicting heart weight to body weight ratios; FIG. 6B is a graph depicting contractility; FIG. 6C is a graph depicting BAG3 protein levels; FIG. 6D is an immunoblotting analysis of BAG3 protein levels.

[0019] FIGS. 7A-7F show hemodynamic indices and BAG3 levels in porcine hearts following balloon occlusion. FIG. 7A is a graph depicting ejection fraction; FIG. 7B is a graph depicting fractional shortening; FIG. 7C is a graph depicting end diastolic volume; FIG. 7D is a graph depicting end systolic volume; FIG. 7E is a graph depicting BAG3 protein levels; FIG. 7F is an immunoblotting analysis of BAG3 protein levels.

[0020] FIG. 8 shows the NCBI reference amino acid sequence for BAG3.

DETAILED DESCRIPTION

[0021] The present invention is based, in part, on the inventors' discovery of a novel BAG3 mutation in a family with adult-onset familial dilated cardiomyopathy (FDC). More specifically, the inventors have found that a novel 10 nucleotide deletion segregated in all affected individuals. Moreover, the inventors also found that levels of BAG3 protein were significantly reduced in hearts from unrelated patients with end-stage heart failure compared to non-failing controls. Further, the inventors have shown that, in a murine model of heart failure, administration of an AAV vector expressing BAG3, restored normal ventricular function. Accordingly, the invention features compositions that increase the expression of BAG3, methods of making such compositions, and methods of using such compositions to treat a subject, i.e., a patient suffering from dilated cardiomyopathy. Also featured are methods and compositions for diagnosis of heart failure, for example, idiopathic dilated cardiomyopathy (IDC).

[0022] Bcl-2 associated anthanogene-3 (BAG3), also known as BCL2-Associated Athanogene 3; MFM6; Bcl-2-Binding Protein Bis;CAIR-1; Docking Protein CAIR-1; BAG Family Molecular Chaperone Regulator 3; BAG-3; BCL2-Binding Athanogene 3; or BIS, is a cytoprotective polypeptide that competes with Hip-1 for binding to HSP 70. BAG3 function is illustrated in FIG. 8 and the mechanism for BAG3 involvement in cardiomyocyte function is illustrated in FIG. 9. The NCBI reference amino acid sequence for BAG3 can be found at Genbank under accession number NP_004272.2; Public GI:14043024. We refer to the amino acid sequence of Genbank accession number NP_004272.2; Public GI:14043024 as SEQ ID NO: 1 as shown in FIG. 10. The NCBI reference nucleic acid sequence for BAG3 can be found at Genbank under accession number NM_004281.3 GI:62530382. We refer to the nucleic acid sequence of Genbank accession number NM_004281.3 GI:62530382 as SEQ ID NO: 2. Other BAG3 amino acid sequences include, for example, without limitation, 095817.3 GI:12643665 (SEQ ID NO: 3); EAW49383.1 GI:119569768 (SEQ ID NO: 4); EAW49382.1 GI:119569767(SEQ ID NO: 5); and CAF55998.1 GI:38502170 (SEQ ID NO: 6). The BAG3 polypeptide of the invention can be a variant of a polypeptide described herein, provided it retains functionality.

[0023] Vectors containing nucleic acids encoding a BAG3 polypeptide are provided herein.

[0024] A novel BAG3 mutation was identified in a family with adult-onset FDC. BAG3 protein levels were significantly decreased in unrelated patients with non-familial IDC providing evidence that altered levels of BAG3 protein participate in the progression of HF.

[0025] Embodiments are directed to compositions which modulate expression of Bcl-2 associated anthanogene-3 (BAG3) in vivo or in vitro. Modulation of BAG3 in patients in need of such therapy, include, patients with cardiac diseases or disorders, for example heart failure, or muscular-skeletal diseases or disorders. Embodiments are also directed to identification of novel compounds or agents which modulate BAG3 expression using assays which measure BAG3 expression.

[0026] Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. The present invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention.

[0027] Embodiments of the invention may be practiced without the theoretical aspects presented. Moreover, the theoretical aspects are presented with the understanding that Applicants do not seek to be bound by the theory presented.

[0028] All genes, gene names, and gene products disclosed herein are intended to correspond to homologs from any species for which the compositions and methods disclosed herein are applicable. Thus, the terms include, but are

not limited to genes and gene products from humans and mice. It is understood that when a gene or gene product from a particular species is disclosed, this disclosure is intended to be exemplary only, and is not to be interpreted as a limitation unless the context in which it appears clearly indicates. Thus, for example, for the genes disclosed herein, which in some embodiments relate to mammalian nucleic acid and amino acid sequences are intended to encompass homologous and/or orthologous genes and gene products from other animals including, but not limited to other mammals, fish, amphibians, reptiles, and birds. In preferred embodiments, the genes or nucleic acid sequences are human.

Definitions

[0029] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[0030] As used herein, the terms "comprising," "comprise" or "comprised," and variations thereof, in reference to defined or described elements of an item, composition, apparatus, method, process, system, etc. are meant to be inclusive or open ended, permitting additional elements, thereby indicating that the defined or described item, composition, apparatus, method, process, system, etc. includes those specified elements--or, as appropriate, equivalents thereof--and that other elements can be included and still fall within the scope/definition of the defined item, composition, apparatus, method, process, system, etc.

[0031] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0032] "Optional" or "optionally" means that the subsequently described circumstance may or may not occur, such that the description includes instances where the circumstance occurs and instances where it does not.

[0033] The term "expression vector" as used herein refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules, siRNA, ribozymes, and the like. Expression vectors can contain a variety of control sequences, which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operatively linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well.

[0034] A "recombinant viral vector" refers to a viral vector comprising one or more heterologous gene products or sequences. Since many viral vectors exhibit size-constraints associated with packaging, the heterologous gene products or sequences are typically introduced by replacing one or more portions of the viral genome. Such viruses may become replication-defective, requiring the deleted function (s) to be provided in trans during viral replication and encapsidation (by using, e.g., a helper virus or a packaging cell line carrying gene products necessary for replication and/or encapsidation). Modified viral vectors in which a polynucleotide to be delivered is carried on the outside of the viral particle have also been described (see, e.g., Curiel, D T, et al., *PNAS* 88: 8850-8854, 1991).

[0035] By "encoding" or "encoded", "encodes", with respect to a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid, or may lack such intervening non-translated sequences (e.g., as in cDNA). The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the "universal" genetic code.

[0036] As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

[0037] A "constitutive promoter" is a promoter which drives expression of a gene to which it is operably linked, in a constant manner in a cell. By way of example, promoters which drive expression of cellular housekeeping genes are considered to be constitutive promoters.

[0038] An "inducible" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only when an inducer which corresponds to the promoter is present in the cell.

[0039] A "tissue-specific" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

[0040] As used herein "BAG3", "BAG3 molecules", "BCL2-associated athanogene 3 (BAG3) genes", "BCL2-associated athanogene 3 (BAG3) molecules" are inclusive of all family members, mutants, cDNA sequences, alleles, fragments, species, coding and noncoding sequences, sense and antisense polynucleotide strands, etc. Similarly "BAG3", "BAG3 molecules", "BCL2-associated athano-

gene 3 (BAG3) molecules" also refer to BAG3 polypeptides or fragment thereof, proteins, variants, derivatives etc. The term "molecule", thus encompasses both the nucleic acid sequences and amino acid sequences of BAG3.

[0041] An "isolated nucleic acid or cDNA" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs, and refers to nucleic acid sequences in which one or more introns have been removed. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA, for instance, DNA which is part of a hybrid gene encoding additional polypeptide sequences.

[0042] A "polynucleotide" means a single strand or parallel and anti-parallel strands of a nucleic acid. Thus, a polynucleotide may be either a single-stranded or a double-stranded nucleic acid.

[0043] The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to a wild type gene. This definition may also include, for example, "allelic," "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. Of particular utility in the invention are variants of wild type gene products. Variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes that give rise to variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

[0044] Unless otherwise indicated, the terms "peptide", "polypeptide" or "protein" are used interchangeably herein, although typically they refer to peptide sequences of varying sizes.

[0045] "Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as "encoding" the protein or other product of that gene or cDNA.

[0046] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

[0047] A "non-natural amino acid" refers to an amino acid that is not one of the 20 common amino acids or pyrolysine or selenocysteine. Other terms that may be used synonymously with the term "non-natural amino acid" is "non-naturally encoded amino acid," "unnatural amino acid," "non-naturally-occurring amino acid," and variously hyphenated and non-hyphenated versions thereof. The term "non-natural amino acid" includes, but is not limited to, amino acids which occur naturally by modification of a naturally encoded amino acid (including but not limited to, the 20 common amino acids or pyrrolysine and selenocysteine) but are not themselves incorporated, without user manipulation, into a growing polypeptide chain by the translation complex. Examples of naturally-occurring amino acids that are not naturally-encoded include, but are not limited to, N-acetylglucosaminyl-L-serine, N-acetylglucosaminyl-L-threonine, and O-phosphotyrosine. Additionally, the term "non-natural amino acid" includes, but is not limited to, amino acids which do not occur naturally and may be obtained synthetically or may be obtained by modification of non-natural amino acids.

[0048] As used herein, the term "misexpression" refers to a non-wild type pattern of gene expression. It includes: expression at non-wild type levels, i.e., over- or underexpression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

[0049] By the term "modulate," it is meant that any of the mentioned activities of the compounds embodied herein, are, e.g., increased, enhanced, increased, agonized (acts as an agonist), promoted, decreased, reduced, suppressed blocked, or antagonized (acts as an antagonist). Modulation can increase activity more than 1-fold, 2-fold, 3-fold, 5-fold, 10-fold, 100-fold, etc., over baseline values. Modulation can also decrease its activity below baseline values.

[0050] As used herein, the term "agent" is meant to encompass any molecule, chemical entity, composition, drug, therapeutic agent, chemotherapeutic agent, or biologi-

cal agent capable of preventing, ameliorating, or treating a disease or other medical condition. The term includes small molecule compounds, antisense reagents, siRNA reagents, antibodies, enzymes, peptides organic or inorganic molecules, natural or synthetic compounds and the like. An agent can be assayed in accordance with the methods of the invention at any stage during clinical trials, during pre-trial testing, or following FDA-approval.

[0051] As defined herein, a "therapeutically effective" amount of a compound or agent (i.e., an effective dosage) means an amount sufficient to produce a therapeutically (e.g., clinically) desirable result. The compositions can be administered one from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the compounds of the invention can include a single treatment or a series of treatments.

[0052] The terms "determining", "measuring", "evaluating", "detecting", "assessing" and "assaying" are used interchangeably herein to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assessing may be relative or absolute. "Assessing the presence of" includes determining the amount of something present, as well as determining whether it is present or absent.

[0053] The term "assay" used herein, whether in the singular or plural shall not be misconstrued or limited as being directed to only one assay with specific steps but shall also include, without limitation any further steps, materials, various iterations, alternatives etc., that can also be used. Thus, if the term "assay" is used in the singular, it is merely for illustrative purposes.

[0054] A "label" or a "detectable label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radio labeled molecules fluorophores, luminescent compounds, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be made detectable, e.g., by incorporating a label into the peptide or used to detect antibodies specifically reactive with the peptide.

[0055] The term "high-throughput screening" or "HTS" refers to a method drawing on different technologies and disciplines, for example, optics, chemistry, biology or image analysis to permit rapid, highly parallel biological research and drug discovery. HTS methods are known in the art and they are generally performed in multiwell plates with automated liquid handling and detection equipment; however it is envisioned that the methods of the invention may be practiced on a microarray or in a microfluidic system.

[0056] The term "library" or "drug library" as used herein refers to a plurality of chemical molecules (test compound), a plurality of nucleic acids, a plurality of peptides, or a plurality of proteins, organic or inorganic compounds, synthetic molecules, natural molecules, or combinations thereof

[0057] As used herein, the term "target" or "target molecule" refers to any type of molecule, or structure to be detected, manipulated or characterized. The molecule can be an intracellular molecule, such as for example, nucleic acid sequences, peptides, structures (e.g. intracellular membranes, ribosomes, etc.), surface molecules (e.g. receptors), extracellular molecules (e.g. cytokines, enzymes, viral particles, organisms, biological samples and the like.

[0058] As used herein, "biological samples" include solid and body fluid samples. The biological samples used in the present invention can include cells, protein or membrane extracts of cells, blood or biological fluids such as ascites fluid or brain fluid (e.g., cerebrospinal fluid). Examples of solid biological samples include, but are not limited to, samples taken from tissues of the central nervous system, bone, breast, kidney, cervix, endometrium, head/neck, gallbladder, parotid gland, prostate, pituitary gland, muscle, esophagus, stomach, small intestine, colon, liver, spleen, pancreas, thyroid, heart, lung, bladder, adipose, lymph node, uterus, ovary, adrenal gland, testes, tonsils, thymus and skin, or samples taken from tumors. Examples of "body fluid samples" include, but are not limited to blood, serum, semen, prostate fluid, seminal fluid, urine, feces, saliva, sputum, mucus, bone marrow, lymph, and tears.

[0059] As used herein, "cardiac disease" refers to any type of heart disease including heart failure, heart muscle disease, cardiomyopathy, hypertrophic cardiomyopathy, dilated cardiomyopathy, atherosclerosis, coronary artery disease, ischemic heart disease, myocarditis, viral infection, wounds, hypertensive heart disease, valvular disease, congenital heart disease, myocardial infarction, congestive heart failure, arrhythmias, diseases resulting in remodeling of the heart, etc. Diseases of the heart can be due to any reason, such as for example, damage to cardiac tissue such as a loss of contractility (e.g., as might be demonstrated by a decreased ejection fraction).

[0060] Cardiac damage or disorder characterized by insufficient cardiac function includes any impairment or absence of a normal cardiac function or presence of an abnormal cardiac function. Abnormal cardiac function can be the result of disease, injury, and/or aging. As used herein, abnormal cardiac function includes morphological and/or functional abnormality of a cardiomyocyte, a population of cardiomyocytes, or the heart itself. Non-limiting examples of morphological and functional abnormalities include physical deterioration and/or death of cardiomyocytes, abnormal growth patterns of cardiomyocytes, abnormalities in the physical connection between cardiomyocytes, under- or over-production of a substance or substances by cardiomyocytes, failure of cardiomyocytes to produce a substance or substances which they normally produce, and transmission of electrical impulses in abnormal patterns or at abnormal times. Abnormalities at a more gross level include dyskinesis, reduced ejection fraction, changes as observed by echocardiography (e.g., dilatation), changes in EKG, changes in exercise tolerance, reduced capillary perfusion, and changes as observed by angiography. Abnormal cardiac function is seen with many disorders including, for example, ischemic heart disease, e.g., angina pectoris, myocardial infarction, chronic ischemic heart disease, hypertensive heart disease, pulmonary heart disease (cor pulmonale), valvular heart disease, e.g., rheumatic fever, mitral valve prolapse, calcification of mitral annulus, carcinoid heart disease, infective endocarditis, congenital heart disease, myocardial disease, e.g., myocarditis, dilated cardiomyopathy, hypertensive cardiomyopathy, cardiac disorders which result in congestive heart failure, and tumors of the heart,

e.g., primary sarcomas and secondary tumors. Heart damage also includes wounds, such as for example, knife wound; biological (e.g. viral; autoimmune diseases) or chemical (e.g. chemotherapy, drugs); surgery; transplantation and the like.

[0061] As used herein the phrase "diagnostic" means identifying the presence or nature of a pathologic condition. Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of "true positives"). Diseased individuals not detected by the assay are "false negatives." Subjects who are not diseased and who test negative in the assay are termed "true negatives." The "specificity" of a diagnostic assay is 1 minus the false positive rate, where the "false positive" rate is defined as the proportion of those without the disease who test positive. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

[0062] As used herein the phrase "diagnosing" refers to classifying a disease or a symptom, determining a severity of the disease, monitoring disease progression, forecasting an outcome of a disease and/or prospects of recovery. The term "detecting" may also optionally encompass any of the above. Diagnosis of a disease according to the present invention can be effected by determining a level of a polynucleotide or a polypeptide of the present invention in a biological sample obtained from the subject, wherein the level determined can be correlated with predisposition to, or presence or absence of the disease. It should be noted that a "biological sample obtained from the subject" may also optionally comprise a sample that has not been physically removed from the subject, as described in greater detail below.

[0063] "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology or symptoms of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. "Treatment" may also be specified as palliative care. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. Accordingly, "treating" or "treatment" of a state, disorder or condition includes: (1) preventing or delaying the appearance of clinical symptoms of the state, disorder or condition developing in a human or other mammal that may be afflicted with or predisposed to the state, disorder or condition but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition; (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof (in case of maintenance treatment) or at least one clinical or subclinical symptom thereof; or (3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or subclinical symptoms. The benefit to an individual to be treated is either statistically significant or at least perceptible to the patient or to the physician.

[0064] The terms "patient" or "individual" or "subject" are used interchangeably herein, and refers to a mammalian subject to be treated, with human patients being preferred. In some cases, the methods of the invention find use in experimental animals, in veterinary application, and in the development of animal models for disease, including, but not limited to, rodents including mice, rats, and hamsters; and primates.

[0065] As used herein, the term "kit" refers to any delivery system for delivering materials. In the context of reaction assays, such delivery systems include systems that allow for the storage, transport, or delivery of reaction reagents (e.g., oligonucleotides, enzymes, etc. in the appropriate containers) and/or supporting materials (e.g., buffers, written instructions for performing the assay etc.) from one location to another. For example, kits include one or more enclosures (e.g., boxes) containing the relevant reaction reagents and/or supporting materials. As used herein, the term "fragmented kit" refers to a delivery systems comprising two or more separate containers that each contain a subportion of the total kit components. The containers may be delivered to the intended recipient together or separately. For example, a first container may contain an enzyme for use in an assay, while a second container contains oligonucleotides. The term "fragmented kit" is intended to encompass kits containing Analyte specific reagents (ASR's) regulated under section 520(e) of the Federal Food, Drug, and Cosmetic Act, but are not limited thereto. Indeed, any delivery system comprising two or more separate containers that each contains a subportion of the total kit components are included in the term "fragmented kit." In contrast, a "combined kit" refers to a delivery system containing all of the components of a reaction assay in a single container (e.g., in a single box housing each of the desired components). The term "kit" includes both fragmented and combined kits.

Compositions

[0066] The most common cause of dilated cardiomyopathy and heart failure (HF) is ischemic heart disease, however, in a third of all patients the cause remains undefined and patients are diagnosed as having idiopathic dilated cardiomyopathy (IDC). The studies conducted herein, employed whole-exome sequencing to identify the causative variant in a large family with autosomal dominant transmission of dilated cardiomyopathy. Sequencing and subsequent informatics revealed a novel 10-nucleotide deletion in the BCL2-associated athanogene 3 (BAG3) gene ((Ch10:del 121436332_12143641: del. 1266_1275 [NM 004281]) that segregated with all affected individuals. The deletion predicted a shift in the reading frame with the resultant deletion of 135 amino acids from the C-terminal end of the protein. Consistent with genetic variants in genes encoding other sarcomeric proteins there was a considerable amount of genetic heterogeneity in the affected family members. Interestingly, it was also found that the levels of BAG3 protein were significantly reduced in the hearts from unrelated patients with end-stage HF undergoing cardiac transplantation when compared with non-failing controls. Diminished levels of BAG3 protein may be associated with both familial and non-familial forms of dilated cardiomyopathy. Accordingly, modulation of expression of BAG3 or amounts of BAG3 in a patient would be of great benefit.

[0067] In embodiments, a therapeutic agent for treatment of diseases associated with BAG3 and associated molecules and pathways thereof, modulates the expression or amounts of BAG3 in a cell.

[0068] In some embodiments, compositions comprise nucleic acid sequences of BCL2-associated athanogene 3

(BAG3), including without limitation, cDNA, sense and/or antisense sequences of BAG3.

[0069] In some embodiments, a composition comprises an expression vector having an isolated nucleic acid or cDNA sequence or synthetic nucleic acid sequence, encoding BCL2-associated athanogene 3 (BAG3) molecules. The term "nucleic acid sequence" will be used for the sake of brevity and will include, without limitation, isolated nucleic acid or cDNA sequences, synthesized or synthetic nucleic acid sequences, chimeric nucleic acid sequences, homologs, orthologs, variants, mutants or combinations thereof.

[0070] In some embodiments, a nucleic acid sequence of BAG3 comprises at least about a 50% sequence identity to wild type BAG3 or cDNA sequences thereof. In other embodiments, the BAG3 nucleic acid sequence comprises at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to wild type BAG3 or cDNA sequences thereof.

[0071] In some embodiments, a nucleic acid sequence of BAG3 further comprises one or more mutations, substitutions, deletions, variants or combinations thereof.

[0072] In some embodiments, the homology, sequence identity or complementarity, between a BAG3 nucleic acid sequence comprising one or more mutations, substitutions, deletions, variants or combinations thereof and the native or wild type or cDNA sequences of BAG3 is from about 50% to about 60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

[0073] In one embodiment, an expression vector encodes a BCL2-associated athanogene 3 (BAG3) gene or cDNA sequences thereof, or modified sequences thereof. In one embodiment, the expression vector encodes a nucleic acid sequence comprising at least about 50% sequence identity to wild type BCL2-associated athanogene 3 (BAG3) or cDNA sequences thereof. In other embodiments, the nucleic acid sequence comprises at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to wild type BCL2-associated athanogene 3 (BAG3) or cDNA sequences thereof

[0074] A wide variety of host/expression vector combinations may be employed in expressing the BAG3 DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col E1, pCR1, pBR322, pMal-C2, pET, pGEX (Smith et al., Gene 67:31-40, 1988), pMB9 and their derivatives, plasmids such as RP4; phage DNAs, e.g., the numerous derivatives of phage 1, e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof, vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

[0075] A number of vectors are known to be capable of mediating transfer of gene products to mammalian cells, as is known in the art and described herein. A "vector" (sometimes referred to as gene delivery or gene transfer "vehicle") refers to a macromolecule or complex of molecules comprising a polynucleotide to be delivered to a host cell, either in vitro or in vivo. The polynucleotide to be delivered may comprise a coding sequence of interest in gene therapy. Vectors include, for example, viral vectors (such as adenoviruses ("Ad"), adeno-associated viruses (AAV), and vesicular stomatitis virus (VSV) and retroviruses), liposomes and other lipid-containing complexes, and other macromolecular complexes capable of mediating delivery of a polynucleotide to a host cell. Vectors can also comprise other components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. As described and illustrated in more detail below, such other components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector nucleic acid by the cell; components that influence localization of the polynucleotide within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of the polynucleotide. Such components also might include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities. Other vectors include those described by Chen et al; *BioTechniques*, 34: 167-171 (2003). A large variety of such vectors are known in the art and are generally available.

[0076] Suitable nucleic acid delivery systems include viral vector, typically sequence from at least one of an adenovirus, adenovirus-associated virus (AAV), helper-dependent adenovirus, retrovirus, or hemagglutinating virus of Japan-liposome (HVJ) complex. Preferably, the viral vector comprises a strong eukaryotic promoter operably linked to the polynucleotide e.g., a cytomegalovirus (CMV) promoter.

[0077] Additionally preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include Moloney murine leukemia viruses and HIV-based viruses. One HIV-based viral vector comprises at least two vectors wherein the gag and pol genes are from an HIV genome and the env gene is from another virus. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex 1 virus (HSV) vector [Geller, A. I. et al., *J. Neurochem*, 64: 487 (1995); Lim, F., et al., in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); Geller, A. I. et al., *Proc Natl. Acad. Sci.*: U.S.A.:90 7603 (1993); Geller, A. I., et al., *Proc Natl. Acad. Sci* USA: 87:1149 (1990)], Adenovirus Vectors [LeGal LaSalle et al., *Science*, 259:988 (1993); Davidson, et al., *Nat. Genet.* 3: 219 (1993); Yang, et al., *J. Virol.* 69: 2004 (1995)] and Adeno-associated Virus Vectors [Kaplitt, M. G., et al., *Nat. Genet.* 8:148 (1994)].

[0078] Pox viral vectors introduce the gene into the cells cytoplasm. Avipox virus vectors result in only a short term expression of the nucleic acid. Adenovirus vectors, adeno-

associated virus vectors and herpes simplex virus (HSV) vectors may be an indication for some invention embodiments. The adenovirus vector results in a shorter term expression (e.g., less than about a month) than adeno-associated virus (AAV), in some embodiments, may exhibit much longer expression. In some embodiments, the expression vector is an AAV9 vector. The particular vector chosen will depend upon the target cell and the condition being treated. The selection of appropriate promoters can readily be accomplished. Preferably, one would use a high expression promoter. An example of a suitable promoter is the 763-base-pair cytomegalovirus (CMV) promoter. The Rous sarcoma virus (RSV) (Davis, et al., *Hum Gene Ther* 4:151 (1993)) and MMT promoters may also be used. Certain proteins can expressed using their native promoter. Other elements that can enhance expression can also be included such as an enhancer or a system that results in high levels of expression such as a tat gene and tar element. This cassette can then be inserted into a vector, e.g., a plasmid vector such as, pUC19, pUC118, pBR322, or other known plasmid vectors, that includes, for example, an *E. coli* origin of replication. See, Sambrook, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory press, (1989). The plasmid vector may also include a selectable marker such as the β-lactamase gene for ampicillin resistance, provided that the marker polypeptide does not adversely affect the metabolism of the organism being treated. The cassette can also be bound to a nucleic acid binding moiety in a synthetic delivery system, such as the system disclosed in WO 95/22618.

[0079] If desired, the polynucleotides of the invention may also be used with a microdelivery vehicle such as cationic liposomes and adenoviral vectors. For a review of the procedures for liposome preparation, targeting and delivery of contents, see Mannino and Gould-Fogerite, *BioTechniques*, 6:682 (1988). See also, Felgner and Holm, *Bethesda Res. Lab. Focus*, 11(2):2J (1989) and Maurer, R. A., *Bethesda Res. Lab. Focus*, 11(2):25 (1989).

[0080] Replication-defective recombinant adenoviral vectors, can be produced in accordance with known techniques. See, Quantin, et al., *Proc. Natl. Acad. Sci.* USA, 89:2581-2584 (1992); Stratford-Perricadet, et al., J. Clin. Invest., 90:626-630 (1992); and Rosenfeld, et al., Cell, 68:143-155 (1992).

[0081] Another delivery method is to use single stranded DNA producing vectors which can produce the BAG3 intracellularly, for example, cardiac tissues. See for example, Chen et al, *BioTechniques*, 34: 167-171 (2003), which is incorporated herein, by reference, in its entirety.

[0082] Expression of BAG3 may be controlled by any promoter/enhancer element known in the art, but these regulatory elements must be functional in the host selected for expression. In some embodiments, the promoter is a tissue specific promoter. Of particular interest are muscle specific promoters, and more particularly, cardiac specific promoters. These include the myosin light chain-2 promoter (Franz et al. (1994) *Cardioscience*, Vol. 5(4):235-43; Kelly et al. (1995) *J. Cell Biol., Vol.* 129(2):383-396), the alpha actin promoter (Moss et al. (1996) *Biol. Chem., Vol.* 271 (49):31688-31694), the troponin 1 promoter (Bhaysar et al. (1996) *Genomics*, Vol. 35(1):11-23); the Na^+/Ca^{2+} exchanger promoter (Barnes et al. (1997) *J. Biol. Chem.*, Vol. 272(17):11510-11517), the dystrophin promoter (Kimura et al. (1997) *Dev. Growth Differ.*, Vol. 39(3):257-265), the alpha7 integrin promoter (Ziober and Kramer (1996) *J. Bio. Chem.*, Vol. 271(37):22915-22), the brain natriuretic peptide promoter (LaPointe et al. (1996) *Hypertension, Vol.* 27(3 Pt 2):715-22) and the alpha B-crystallin/small heat shock protein promoter (Gopal-Srivastava (1995) *J. Mol. Cell. Biol.*, Vol. 15(12):7081-7090), alpha myosin heavy chain promoter (Yamauchi-Takihara et al. (1989) *Proc. Natl. Acad. Sci.* USA, Vol. 86(10):3504-3508) and the ANF promoter (LaPointe et al. (1988) *J. Biol. Chem.*, Vol. 263(19):9075-9078).

[0083] Other promoters which may be used to control BAG3 gene expression include, but are not limited to, cytomegalovirus (CMV) promoter (U.S. Pat. Nos. 5,385,839 and 5,168,062), the SV40 early promoter region (Benoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., *Cell* 22:787-797, 1980), the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci.* U.S.A. 78:1441-1445, 1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39-42, 1982); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., *Proc. Natl. Acad. Sci.* U.S.A. 75:3727-3731, 1978), or the tac promoter (DeBoer, et al., *Proc. Natl. Acad. Sci.* U.S.A. 80:21-25, 1983); see also "Useful proteins from recombinant bacteria" in Scientific American, 242:74-94, 1980; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., *Cell* 38:639-646, 1984; Ornitz et al., Cold Spring Harbor Symp. *Quant. Biol.* 50:399-409, 1986; MacDonald, *Hepatology* 7:425-515, 1987); insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature* 315:115-122, 1985), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., *Cell* 38:647-658, 1984; Adames et al., *Nature* 318:533-538, 1985; Alexander et al., *Mol. Cell. Biol.* 7:1436-1444, 1987), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., *Cell* 45:485-495, 1986), albumin gene control region which is active in liver (Pinkert et al., *Genes and Devel.* 1:268-276, 1987), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., *Mol. Cell. Biol.* 5:1639-1648, 1985; Hammer et al., *Science* 235:53-58, 1987), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., *Genes and Devel.* 1: 161-171, 1987), beta-globin gene control region which is active in myeloid cells (Mogram et al., *Nature* 315:338-340, 1985; Kollias et al., *Cell* 46:89-94, 1986), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., *Cell* 48:703-712, 1987), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature* 314:283-286, 1985), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., *Science* 234:1372-1378, 1986).

[0084] Yeast expression systems can also be used according to the invention to express BAG3. For example, the non-fusion pYES2 vector (XbaI, SphI, ShoI, NotI, GstXI, EcoRI, BstXI, BamHI, SacI, KpnI, and HindIII cloning sites; Invitrogen) or the fusion pYESHisA, B, C (XbaI, SphI,

ShoI, NotI, BstXI, EcoRI, BamHI, SacI, KpnI, and HindIII cloning sites, N-terminal peptide purified with ProBond resin and cleaved with enterokinase; Invitrogen), to mention just two, can be employed according to the invention. A yeast two-hybrid expression system can be prepared in accordance with the invention.

[0085] One preferred delivery system is a recombinant viral vector that incorporates one or more of the polynucleotides therein, preferably about one polynucleotide. Preferably, the viral vector used in the invention methods has a pfu (plague forming units) of from about 10^8 to about 5×10^{10} pfu. In embodiments in which the polynucleotide is to be administered with a non-viral vector, use of between from about 0.1 nanograms to about 4000 micrograms will often be useful e.g., about 1 nanogram to about 100 micrograms.

[0086] In some embodiments, the vector is an adenovirus-associated viral vector (AAV), for example, AAV9. The term "AAV vector" means a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7 and AAV-8. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes, but retain functional flanking ITR sequences. Despite the high degree of homology, the different serotypes have tropisms for different tissues. The receptor for AAV1 is unknown; however, AAV1 is known to transduce skeletal and cardiac muscle more efficiently than AAV2. Since most of the studies have been done with pseudotyped vectors in which the vector DNA flanked with AAV2 ITR is packaged into capsids of alternate serotypes, it is clear that the biological differences are related to the capsid rather than to the genomes. Recent evidence indicates that DNA expression cassettes packaged in AAV 1 capsids are at least 1 log 10 more efficient at transducing cardiomyocytes than those packaged in AAV2 capsids. In one embodiment, the viral delivery system is an adeno-associated viral delivery system. The adeno-associated virus can be of serotype 1 (AAV 1), serotype 2 (AAV2), serotype 3 (AAV3), serotype 4 (AAV4), serotype 5 (AAV5), serotype 6 (AAV6), serotype 7 (AAV7), serotype 8 (AAV8), or serotype 9 (AAV9).

[0087] Some skilled in the art have circumvented some of the limitations of adenovirus-based vectors by using adenovirus "hybrid" viruses, which incorporate desirable features from adenovirus as well as from other types of viruses as a means of generating unique vectors with highly specialized properties. For example, viral vector chimeras were generated between adenovirus and adeno-associated virus (AAV). These aspects of the invention do not deviate from the scope of the invention described herein.

[0088] Nucleic acids encoding the BAG3 proteins of the invention may be delivered to cardiac muscle by methods known in the art (see e.g., US Patent Appln. Publication No. US 2009/0209631). For example, cardiac cells of a large mammal may be transfected by a method that includes dilating a blood vessel of the coronary circulation by administering a vasodilating substance to said mammal prior to, and/or concurrent with, administering the nucleic acids. In some embodiments, the method includes administering the nucleic acids into a blood vessel of the coronary circulation in vivo, wherein nucleic acids are infused into the blood vessel over a period of at least about three minutes, wherein the coronary circulation is not isolated or substantially isolated from the systemic circulation of the mammal, and wherein the nucleic acids transfect cardiac cells of the mammal.

[0089] In some embodiments, the subject can be a human, an experimental animal, e.g., a rat or a mouse, a domestic animal, e.g., a dog, cow, sheep, pig or horse, or a non-human primate, e.g., a monkey. The subject may be suffering from a cardiac disorder, such as heart failure, ischemia, myocardial infarction, congestive heart failure, arrhythmia, transplant rejection and the like. In a preferred embodiment, the subject is suffering from heart failure. In another particular embodiment, the subject is suffering from arrhythmia. In one embodiment, the subject is a human. For example, the subject is between ages 18 and 65. In another embodiment, the subject is a non-human animal.

[0090] In one embodiment, the subject has or is at risk for heart failure, e.g. a non-ischemic cardiomyopathy, mitral valve regurgitation, ischemic cardiomyopathy, or aortic stenosis or regurgitation.

[0091] In some embodiments, transfection of cardiac cells with nucleic acid molecules encoding a BAG3 protein or BAG3 protein fused to an effector domain increases lateral ventricle fractional shortening. In some embodiments, the mammal is human and the disease is congestive heart failure. In some embodiments, the transfection of the cardiac cells increases lateral ventricle fractional shortening when measured about 4 months after said infusion by at least 25% as compared to lateral ventricle fractional shortening before infusion of the polynucleotide. In some embodiments, the transfection of the cardiac cells results in an improvement in a measure of cardiac function selected from the group consisting of expression of BAG3 protein, fractional shortening, ejection fraction, cardiac output, time constant of ventricular relaxation, and regurgitant volume.

[0092] A treatment can be evaluated by assessing the effect of the treatment on a parameter related to contractility. For example, SR Ca^{2+} ATPase activity or intracellular Ca^{2+} concentration can be measured. Furthermore, force generation by hearts or heart tissue can be measured using methods described in Strauss et al., *Am. J. Physiol.*, 262:1437-45, 1992, the contents of which are incorporated herein by reference.

[0093] Modified Nucleic Acid Sequences: It is not intended that the present invention be limited by the nature of the nucleic acid employed, as long as they modulate the expression or quantities of BAG3 in a cell, or patient to whom, the nucleic acid composition is to be administered as a therapeutic agent. The nucleic acid may be DNA or RNA and may exist in a double-stranded, single-stranded or partially double-stranded form.

[0094] Nucleic acids useful in the present invention include, by way of example and not limitation, oligonucleotides and polynucleotides such as antisense DNAs and/or RNAs; ribozymes; DNA for gene therapy; viral fragments including viral DNA and/or RNA; DNA and/or RNA chimeras; mRNA; plasmids; cosmids; genomic DNA; cDNA; gene fragments; various structural forms of DNA including single-stranded DNA, double-stranded DNA, supercoiled DNA and/or triple-helical DNA; Z-DNA; and the like. The nucleic acids may be prepared by any conventional means typically used to prepare nucleic acids in large quantity. For example, DNAs and RNAs may be chemically synthesized using commercially available reagents and synthesizers by methods that are well-known in the art (see, e.g., Gait, 1985,

OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH (IRL Press, Oxford, England)). RNAs may be produce in high yield via in vitro transcription using plasmids such as pGEM® T vector or SP65 (Promega Corporation, Madison, Wis.).

[0095] Accordingly, certain preferred nucleic acid sequences of this invention are chimeric nucleic acid sequences. "Chimeric nucleic acid sequences" or "chimeras," in the context of this invention, contain two or more chemically distinct regions, each made up of at least one nucleotide. These sequences typically contain at least one region of modified nucleotides that confers one or more beneficial properties (such as, for example, increased nuclease resistance, increased uptake into cells, increased binding affinity for the target).

[0096] Chimeric nucleic acid sequences of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures comprise, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

[0097] Specific examples of some modified nucleic acid sequences envisioned for this invention include those comprising modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Examples of oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, include without limitation: CH_2—NH—O—CH_2, CH, —N(CH_3)—O—CH_2 [known as a methylene(methylimino) or MMI backbone], CH_2—O—N(CH_3)—CH_2, CH_2—N(CH_3)—N(CH_3)—CH_2 and O—N(CH_3)—CH_2—CH_2 backbones, wherein the native phosphodiester backbone is represented as O—P—O—CH_2). The amide backbones disclosed by De Mesmaeker et al. (1995) *Acc. Chem. Res.* 28:366-374 are also one example. In other embodiments, a nucleic acid sequence comprises morpholino backbone structures (Summerton and Weller, U.S. Pat. No. 5,034,506). In other embodiments, such as the peptide nucleic acid (PNA) backbone, the phosphodiester backbone of the nucleic acid sequence is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone (Nielsen et al. (1991) *Science* 254, 1497). Nucleic acid sequences may also comprise one or more substituted sugar moieties. Examples include: OH, SH, SCH_3, F, OCN, OCH_3, OCH_3, $OCH_3O(CH_2)_nCH_3$, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$ where n is from 1 to about 10; C_1 to C_{10} lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF_3; OCF_3; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; $SOCH_3$; SO_2CH_3; ONO_2; NO2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. Other modifications include, for example: 2'-methoxyethoxy [2'-O—$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl)] (Martin et al., (1995) *Helv. Chim. Acta*, 78, 486), 2'-methoxy (2'-O—CH_3), 2'-propoxy (2'-$OCH_2CH_2CH_3$) and 2'-fluoro (2'-F). Similar modifications may also be made at any positions on the oligonucleotide, the 2' or the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. The nucleic acid sequences may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

[0098] Preferred modified oligonucleotide backbones comprise, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3' alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[0099] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[0100] The nucleic acid sequences may also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleotides include adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U). Modified nucleotides include nucleotides found only infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, as well as synthetic nucleotides, e.g., 2-aminoadenine, 2-(methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalklyamino)adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N6 (6-aminohexyl)adenine and 2,6-diaminopurine. (Kornberg, A., DNA Replication, W.H. Freeman & Co., San Francisco, 1980, pp 75-77; Gebeyehu, G., (1987) et al. *Nucl. Acids Res.* 15:4513). A "universal" base known in the art, e.g., inosine, may be included.

[0101] Another modification involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, a cholesteryl moiety, cholic acid, a thioether, e.g., hexyl-5-tritylthiol, an aliphatic

chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid. Nucleic acid sequences comprising lipophilic moieties, and methods for preparing such oligonucleotides are known in the art, for example, U.S. Pat. Nos. 5,138,045, 5,218,105 and 5,459,255.

[0102] It is not necessary for all positions in a given nucleic acid sequence to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single nucleic acid sequence or even at within a single nucleoside within an such sequences. The present invention also includes oligonucleotides which are chimeric oligonucleotides as hereinbefore defined.

[0103] In another embodiment, the BAG3 nucleic acid molecule of the present invention is conjugated with another moiety including but not limited to abasic nucleotides, polyether, polyamine, polyamides, peptides, carbohydrates, lipid, or polyhydrocarbon compounds. Those skilled in the art will recognize that these molecules can be linked to one or more of any nucleotides comprising the nucleic acid molecule at several positions on the sugar, base or phosphate group.

[0104] In another embodiment, the BAG3 nucleic acid sequences comprise one or more nucleotides substituted with locked nucleic acids (LNA). The LNA modified nucleic acid sequences may have a size similar to the parent or native sequence or may be larger or preferably smaller. It is preferred that such LNA-modified oligonucleotides contain less than about 70%, more preferably less than about 60%, most preferably less than about 50% LNA monomers and that their sizes are between about 1 and 25 nucleotides.

[0105] Antisense BAG3-Oligonucleotides: In another preferred embodiment, the expression of BAG3 in a cell or patient is modulated by one or more target nucleic acid sequences which modulate the expression of BAG3, for example, transcriptional regulator elements.

[0106] In a preferred embodiment, an oligonucleotide comprises at least five consecutive bases complementary to a nucleic acid sequence, wherein the oligonucleotide specifically hybridizes to and modulates expression of BAG3 in vivo or in vitro. In another preferred embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenosine, variants may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the oligonucleotide. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

[0107] In some embodiments, homology, sequence identity or complementarity, between the oligonucleotide and target is from about 50% to about 60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

[0108] In another preferred embodiment, an oligonucleotide comprises combinations of phosphorothioate internucleotide linkages and at least one internucleotide linkage selected from the group consisting of: alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and/or combinations thereof.

[0109] In another preferred embodiment, an oligonucleotide optionally comprises at least one modified nucleobase comprising, peptide nucleic acids, locked nucleic acid (LNA) molecules, analogues, derivatives and/or combinations thereof.

[0110] An oligonucleotide is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target nucleic acid sequences under conditions in which specific binding is desired. Such conditions include, i.e., physiological conditions in the case of in vivo assays or therapeutic treatment, and conditions in which assays are performed in the case of in vitro assays.

[0111] An oligonucleotide, whether DNA, RNA, chimeric, substituted etc, is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarily to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

[0112] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

[0113] In embodiments of the present invention oligomeric oligonucleotides, particularly oligonucleotides, bind to target nucleic acid molecules and modulate the expression of molecules encoded by a target gene. The functions of DNA to be interfered comprise, for example, replication and transcription. The functions of RNA to be interfered comprise all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The functions may be up-regulated or inhibited depending on the functions desired.

[0114] The oligonucleotides, include, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

[0115] Targeting an oligonucleotide to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. This target nucleic acid may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state.

[0116] The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, e.g., modulation of expression, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in the present invention, are defined as positions within a target nucleic acid.

[0117] In another preferred embodiment, the antisense oligonucleotides bind to coding and/or non-coding regions of a target polynucleotide and modulate the expression and/or function of the target molecule.

[0118] In another preferred embodiment, the antisense oligonucleotides bind to natural antisense polynucleotides and modulate the expression and/or function of the target molecule. An example of a "function" can be one which inhibits a negative regulator of transcription, thus allowing for an increased expression of a desired molecule, such as, for example, BAG3.

[0119] In another preferred embodiment, the antisense oligonucleotides bind to sense polynucleotides and modulate the expression and/or function of the target molecule.

[0120] In embodiments of the invention the oligonucleotides bind to an antisense strand of a particular target. The oligonucleotides are at least 5 nucleotides in length and can be synthesized so each oligonucleotide targets overlapping sequences such that oligonucleotides are synthesized to cover the entire length of the target polynucleotide. The targets also include coding as well as non coding regions.

[0121] According to the present invention, antisense compounds include antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleobase positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines, however, in preferred embodiments, the gene expression is up regulated. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

[0122] In another preferred embodiment, the desired oligonucleotides or antisense compounds, comprise at least one of: antisense RNA, antisense DNA, chimeric antisense oligonucleotides, antisense oligonucleotides comprising modified linkages, interference RNA (RNAi), short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof.

[0123] dsRNA can also activate gene expression, a mechanism that has been termed "small RNA-induced gene activation" or RNAa. dsRNAs targeting gene promoters induce potent transcriptional activation of associated genes. RNAa was demonstrated in human cells using synthetic dsRNAs, termed "small activating RNAs" (saRNAs).

[0124] Small double-stranded RNA (dsRNA) may also act as small activating RNAs (saRNA). Without wishing to be bound by theory, by targeting sequences in gene promoters, saRNAs would induce target gene expression in a phenomenon referred to as dsRNA-induced transcriptional activation (RNAa).

[0125] In some embodiments, the ribonucleic acid sequence is specific for regulatory segments of the genome that control the transcription of BAG3. Thus a candidate therapeutic agent can be a dsRNA that activates the expression of BAG3 in a cell and is administered to a patient in need of treatment.

[0126] Peptides: In another embodiment, a BAG3 peptide is encoded by a nucleic acid comprising a BCL2-associated athanogene 3 (BAG3) wild type, chimeric or cDNA sequences thereof. The peptide can also be a synthetic peptide of BCL2-associated athanogene 3 (BAG3).

[0127] It is to be understood that the peptide sequences are not limited to the native or cDNA sequences thereof, of BCL2-associated athanogene 3 (BAG3) molecules. The skilled artisan will recognize that conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function. Conservative amino acid substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, lysine, arginine, phenylalanine, tyrosine.

[0128] Conservative substitutions may also be made based on types of amino acids: aliphatic (valine, isoleucine, leu-

cine, and alanine); charged (aspartic acid, glutamic acid, lysine, arginine, and histidine); aromatic residues (phenylalanine, tyrosine and tryptophan); and sulfur-containing (methionine and cysteine). Polypeptide sequences having at least about 68% identity, at least about 70% identity, or at least about 71% identity to a BCL2-associated athanogene 3 (BAG3) nucleic acid sequence, or cDNA sequences thereof.

[0129] The determination of percent identity between two nucleotide or amino acid sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, *Proc. Natl. Acad. Sci.* USA 87:2264-2268), modified as in Karlin and Altschul (1993, *Proc. Natl. Acad. Sci.* USA 90:5873-5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, *J. Mol. Biol.* 215:403-410), and can be accessed, for example at the National Center for Biotechnology Information (NCBI) world wide web site having the universal resource locator http://blast(dot)ncbi(dot)nlm(dot)nih(dot)gov/blast.cgi/. BLAST nucleotide searches can be performed with the NBLAST program (designated "blastn" at the NCBI web site), using the following parameters: gap penalty=5; gap extension penalty=2; mismatch penalty=3; match reward=1; expectation value 10.0; and word size=11 to obtain nucleotide sequences homologous to a nucleic acid described herein. BLAST protein searches can be performed with the XBLAST program or the NCBI "blastp" program, using the following parameters: expectation value 10.0, BLOSUM62 scoring matrix to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997, *Nucleic Acids Res.* 25:3389-3402). Alternatively, PSI-Blast or PHI-Blast can be used to perform an iterated search which detects distant relationships between molecules and relationships between molecules which share a common pattern. When utilizing BLAST, Gapped BLAST, PSI-Blast, and PHI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. In calculating percent identity, exact matches are typically counted.

[0130] Embodiments of the invention also include polynucleotides encoding hybrid proteins comprising BCL2-associated athanogene 3 (BAG3) polypeptide operatively fused directly or indirectly via peptide linker, to a second polypeptide sequence. Linker sequences are well known in the art. In one embodiment, a hybrid protein comprises a BAG3 polypeptide or a BAG3 polypeptide operatively fused to a detectable moiety, such as, a reporter polypeptide, wherein the reporter polypeptide is fused to the N- or C-terminal of the BAG3 polypeptide, directly or indirectly. Exemplary reporter polypeptides include luciferase (LUC), green fluorescent protein (GFP), and GFP derivatives.

[0131] Hybrid proteins comprising a BAG3 polypeptide or fragment thereof may be linked to other types of polypeptides, in addition to a reporter polypeptide, or in lieu of a reporter polypeptide. These additional polypeptides may be any amino acid sequence useful for the purification, identification, and/or therapeutic or prophylactic application of the peptide. In addition, the additional polypeptide can be a signal peptide, or targeting peptide, etc.

[0132] In some cases, the other additions, substitutions or deletions may increase the stability (including but not limited to, resistance to proteolytic degradation) of the polypeptide or increase affinity of the polypeptide for its appropriate receptor, ligand and/or binding proteins. In some cases, the other additions, substitutions or deletions may increase the solubility of the polypeptide. In some embodiments sites are selected for substitution with a naturally encoded or non-natural amino acid in addition to another site for incorporation of a non-natural amino acid for the purpose of increasing the polypeptide solubility following expression in recombinant host cells. In some embodiments, the polypeptides comprise another addition, substitution, or deletion that modulates affinity for the associated ligand, binding proteins, and/or receptor, modulates (including but not limited to, increases or decreases) receptor dimerization, stabilizes receptor dimers, modulates circulating half-life, modulates release or bio-availability, facilitates purification, or improves or alters a particular route of administration. Similarly, the non-natural amino acid polypeptide can comprise chemical or enzyme cleavage sequences, protease cleavage sequences, reactive groups, antibody-binding domains (including but not limited to, FLAG or poly-His) or other affinity based sequences (including but not limited to, FLAG, poly-His, GST, etc.) or linked molecules (including but not limited to, biotin) that improve detection (including but not limited to, GFP), purification, transport through tissues or cell membranes, prodrug release or activation, size reduction, or other traits of the polypeptide.

[0133] The methods and compositions described herein include incorporation of one or more non-natural amino acids into a polypeptide. One or more non-natural amino acids may be incorporated at one or more particular positions which does not disrupt activity of the polypeptide. This can be achieved by making "conservative" substitutions, including but not limited to, substituting hydrophobic amino acids with non-natural or natural hydrophobic amino acids, bulky amino acids with non-natural or natural bulky amino acids, hydrophilic amino acids with non-natural or natural hydrophilic amino acids) and/or inserting the non-natural amino acid in a location that is not required for activity.

[0134] A variety of biochemical and structural approaches can be employed to select the desired sites for substitution with a non-natural amino acid within the polypeptide. Any position of the polypeptide chain is suitable for selection to incorporate a non-natural amino acid, and selection may be based on rational design or by random selection for any or no particular desired purpose. Selection of desired sites may be based on producing a non-natural amino acid polypeptide (which may be further modified or remain unmodified) having any desired property or activity, including but not limited to agonists, super-agonists, partial agonists, inverse agonists, antagonists, receptor binding modulators, receptor activity modulators, modulators of binding to binder partners, binding partner activity modulators, binding partner conformation modulators, dimer or multimer formation, no change to activity or property compared to the native molecule, or manipulating any physical or chemical property of the polypeptide such as solubility, aggregation, or stability. For example, locations in the polypeptide required for biological activity of a polypeptide can be identified using methods including, but not limited to, point mutation analysis, alanine scanning or homolog scanning methods. Residues other than those identified as critical to biological activity by methods including, but not limited to, alanine or homolog scanning mutagenesis may be good candidates for substitution with a non-natural amino acid depending on the

desired activity sought for the polypeptide. Alternatively, the sites identified as critical to biological activity may also be good candidates for substitution with a non-natural amino acid, again depending on the desired activity sought for the polypeptide. Another alternative would be to make serial substitutions in each position on the polypeptide chain with a non-natural amino acid and observe the effect on the activities of the polypeptide. Any means, technique, or method for selecting a position for substitution with a non-natural amino acid into any polypeptide is suitable for use in the methods, techniques and compositions described herein.

Candidate Agents and Screening Assays

[0135] The compositions embodied herein, can also be applied in the areas of drug discovery and target validation. The present invention comprehends the use of the nucleic acid sequences and peptides embodied herein, in drug discovery efforts to elucidate relationships that exist between Bcl-2 associated anthanogene-3 (BAG3) polynucleotides and a disease state, phenotype, or condition. These methods include detecting or modulating Bcl-2 associated anthanogene-3 (BAG3) polynucleotides comprising contacting a sample, tissue, cell, or organism with a compound, measuring the nucleic acid or protein level of Bcl-2 associated anthanogene-3 (BAG3) polynucleotides and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further compound of the invention.

[0136] The screening assays of the invention suitably include and embody, animal models, cell-based systems and non-cell based systems. The nucleic acid sequences and peptides embodied herein, are used for identifying agents of therapeutic interest, e.g. by screening libraries of compounds or otherwise identifying compounds of interest by any of a variety of drug screening or analysis techniques, or synthesis of novel compounds. The gene, allele, fragment, or oligopeptide thereof employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The measurements are conducted as described in detail in the examples section which follows. In embodiments, screening candidate agents is performed to identify those which modulate the translation of BAG3.

[0137] The assays can be of an in vitro or in vivo format. In vitro formats of interest include cell-based formats, in which contact occurs e.g., by introducing the substrate in a medium, such as an aqueous medium, in which the cell is present. In yet other embodiments, the assay may be in vivo, in which a multicellular organism that includes the cell is employed. Contact of a targeting vector encoding the nucleic acid sequences embodied herein, with the target cell(s) may be accomplished using any convenient protocol. In those embodiments where the target cells are present as part of a multicellular organism, e.g., an animal, the vector is conveniently administered to (e.g., injected into, fed to, etc.) the multicellular organism, e.g., a whole animal, where administration may be systemic or localized, e.g., directly to specific tissue(s) and/or organ(s) of the multicellular organism.

[0138] Multicellular organisms of interest include, but are not limited to: insects, vertebrates, such as avian species, e.g., chickens; mammals, including rodents, e.g., mice, rates; ungulates, e.g., pigs, cows, horses; dogs, cats, primates, e.g., monkeys, apes, humans; and the like. As such, the target cells of interest include, but are not limited to: insects cells, vertebrate cells, particularly avian cells, e.g., chicken cells; mammalian cells, including murine, porcine, ungulate, ovine, equine, rat, dog, cat, monkey, and human cells; and the like.

[0139] The target cell comprising the BAG3 polynucleotides or BAG3 polypeptides is contacted with a test compound and the translation of BAG3 is evaluated or assessed by detecting the presence or absence of signal from a detectable moiety, for example, luciferase substrate, i.e., by screening the cell (either in vitro or in vivo) for the presence of a luciferase mediated luminescent signal. The detected signal is then employed to evaluate the translational and/or transcriptional activity of BAG3 in the presence of a test agent.

[0140] The luminescent signal may be detected using any convenient luminescent detection device. In certain embodiments, detectors of interest include, but are not limited to: photo-multiplier tubes (PMTs), avalanche photodiodes (APDs), charge-coupled devices (CCDs); complementary metal oxide semiconductors (CMOS detectors) and the like. The detector may be present in a signal detection device, e.g., luminometer, which is capable of detecting the signal once or a number of times over a predetermined period, as desired. Data may be collected in this way at frequent intervals, for example once every 10 ms, over the course of a given assay time period.

[0141] In certain embodiments, the subject methods are performed in a high throughput (HT) format. In the subject HT embodiments of the subject invention, a plurality of different cells are simultaneously assayed or tested. By simultaneously tested is meant that each of the cells in the plurality are tested at substantially the same time. In general, the number of cells that are tested simultaneously in the subject HT methods ranges from about 10 to 10,000, usually from about 100 to 10,000 and in certain embodiments from about 1000 to 5000. A variety of high throughput screening assays for determining the activity of candidate agent are known in the art and are readily adapted to the present invention, including those described in e.g., Schultz (1998) *Bioorg Med Chem Lett* 8:2409-2414; Fernandes (1998) *Curr Opin Chem Biol* 2:597-603; as well as those described in U.S. Pat. No. 6,127,133; the disclosures of which are herein incorporated by reference.

[0142] In some embodiments, a method of screening for agents which modulate translation and/or transcription of Bcl-2 associated anthanogene-3 (BAG3) comprises contacting a BAG3 molecule with an agent wherein the BAG3 molecule comprises an isolated nucleic acid or cDNA sequence of Bcl-2 associated anthanogene-3 (BAG3) operably linked to a detectable moiety, and at least one stop codon between the BAG3 and the detectable moiety; assessing the level of translation of the BAG3 in the absence of a candidate agent to obtain a reference level of translation and/or transcription, assessing the level of translation and/or transcription of BAG3 in the presence of the candidate agent to obtain a test level of translation and/or transcription, wherein the candidate agent is identified as an agent that increases translation and/or transcription if the test level of translation and/or transcription is greater than the reference level of translation and/or transcription.

[0143] In embodiments, the detectable moiety comprises: a luminescent moiety, a chemiluminescent moiety, a fluorescence moiety, a bioluminescent moiety, an enzyme, a natural or synthetic moiety.

[0144] Any method known in the art can be used to assess translation. In a preferred embodiment, translation is assessed using mammalian cells transfected with an expression vector comprising a nucleic acid of the invention. The transfection may be transient or the cells may stably transformed with the expression vector. A cell-based assay such as described in Butcher et al., 2007, *J Biol Chem.* 282:2853-28539 may be used. Alternatively, an in vitro translation assay may be used.

[0145] In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast or insect cell, by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical or biological means.

[0146] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, photoporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

[0147] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0148] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (i.e., an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

[0149] In the case where a non-viral delivery system is utilized, a preferred delivery vehicle is a liposome. The above-mentioned delivery systems and protocols therefore can be found in "Gene Targeting Protocols, 2ed.", Kmiec ed., Humana Press, Totowa, N.J., pp 1-35 (2002) and "Gene Transfer and Expression Protocols, Vol. 7, (Methods in Molecular Biology)," Murray ed., Humana Press, Totowa, N.J., pp 81-89 (1991).

[0150] Candidate Agents: The methods can be practiced with any test compounds as candidate agents. Test compounds useful in practicing the inventive method may be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries, spatially-addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, nonpeptide oligomer, or small molecule libraries of compounds (Lam, 1997, *Anticancer Drug Des.* 12:145).

[0151] Examples of methods for the synthesis of molecular libraries may be found in the art, for example, in: DeWitt et al., 1993, Proc. Natl. Acad. Sci. USA 90:6909-6913; Erb et al., 1994, *Proc. Natl. Acad. Sci.* USA 91:11422-11426; Zuckermann et al., 1994, *J. Med. Chem.* 37:2678-2685; Cho et al., 1992, *Science* 261:1303-1305; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2059-2061; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2061-2064; and Gallop et al., 1994, *J. Med. Chem.* 37:1233-1251.

[0152] Libraries of compounds may be presented in solution (e.g., Houghten, 1992, *Bio/Techniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria (U.S. Pat. No. 5,223,409), spores (U.S. Pat. Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al., 1992, *Proc. Natl. Acad. Sci.* USA 89:1865-1869), or phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, *Science* 249:404-406; Cwirla et al., 1990, *Proc. Natl. Acad. Sci.* USA 87:6378-6382; and Felici, 1991, *J Mol. Biol.* 222:301-310).

[0153] Commercially available libraries that may be screened include, but are not limited to, the TimTec Natural Product Library (NPL), NPL-640, and TimTec NDL-3000 library. Libraries comprising compounds modeled on polyamines (i.e., polyamine analogs) may also be screened.

[0154] In certain embodiments, the candidate agent is a small molecule or large molecule ligand. By small molecule ligand is meant a ligand ranging in size from about 50 to about 10,000 daltons, usually from about 50 to about 5,000 daltons and more usually from about 100 to about 1000 daltons. By large molecule is meant a ligand ranging in size from about 10,000 daltons or greater in molecular weight.

[0155] The method may be practiced iteratively using different concentrations of a test candidate and/or different testing conditions, such as duration of reaction time. Test candidates that are identified by the method can be further tested by conventional methods in the art to verify specificity, dose dependency, efficacy in vivo, and the like. Test candidates may serve as lead compounds for developing additional test candidates.

[0156] As indicated above, the present invention finds use in monitoring translational and/or transcriptional activity of BAG3 in an assay wherein the test is conducted using cells. In these embodiments, the cells are cultured under specific user-defined conditions (e.g., in the presence or absence of a cytokine, nutrient and/or candidate therapeutic agent), and monitored for emitted light.

[0157] A prototype compound or agent may be believed to have therapeutic activity on the basis of any information available to the artisan. For example, a prototype agent may be believed to have therapeutic activity on the basis of information contained in the Physician's Desk Reference. In addition, by way of non-limiting example, a compound may be believed to have therapeutic activity on the basis of experience of a clinician, structure of the compound, structural activity relationship data, EC_{50}, assay data, IC_{50} assay data, animal or clinical studies, or any other basis, or combination of such bases.

[0158] A therapeutically-active compound or agent is an agent that has therapeutic activity, including for example, the ability of the agent to induce a specified response when administered to a subject or tested in vitro. Therapeutic

activity includes treatment of a disease or condition, including both prophylactic and ameliorative treatment. Treatment of a disease or condition can include improvement of a disease or condition by any amount, including prevention, amelioration, and elimination of the disease or condition. Therapeutic activity may be conducted against any disease or condition, including in a preferred embodiment against any disease or disorder associated with damage by reactive oxygen intermediates. In order to determine therapeutic activity any method by which therapeutic activity of a compound may be evaluated can be used. For example, both in vivo and in vitro methods can be used, including for example, clinical evaluation, EC_{50}, and IC_{50} assays, and dose response curves.

[0159] Candidate compounds for use with an assay of the present invention or identified by assays of the present invention as useful pharmacological agents can be pharmacological agents already known in the art or variations thereof or can be compounds previously unknown to have any pharmacological activity. The candidate compounds can be naturally occurring or designed in the laboratory. Candidate compounds can comprise a single diastereomer, more than one diastereomer, or a single enantiomer, or more than one enantiomer.

[0160] Candidate compounds can be isolated, from microorganisms, animals or plants, for example, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, candidate compounds of the present invention can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries. The other four approaches are applicable to polypeptide, non-peptide oligomers, or small molecule libraries of compounds and are preferred approaches in the present invention. See Lam, *Anticancer Drug Des.* 12: 145-167 (1997).

[0161] In an embodiment, the present invention provides a method of identifying a candidate compound as a suitable prodrug. A suitable prodrug includes any prodrug that may be identified by the methods of the present invention. Any method apparent to the artisan may be used to identify a candidate compound as a suitable prodrug.

[0162] In another aspect, the present invention provides methods of screening candidate compounds for suitability as therapeutic agents. Screening for suitability of therapeutic agents may include assessment of one, some or many criteria relating to the compound that may affect the ability of the compound as a therapeutic agent. Factors such as, for example, efficacy, safety, efficiency, retention, localization, tissue selectivity, degradation, or intracellular persistence may be considered. In an embodiment, a method of screening candidate compounds for suitability as therapeutic agents is provided, where the method comprises providing a candidate compound identified as a suitable prodrug, determining the therapeutic activity of the candidate compound, and determining the intracellular persistence of the candidate compound. Intracellular persistence can be measured by any technique apparent to the skilled artisan, such as for example by radioactive tracer, heavy isotope labeling, or LCMS.

[0163] In screening compounds for suitability as therapeutic agents, intracellular persistence of the candidate compound is evaluated. In a preferred embodiment, the agents are evaluated for their ability to modulate the translation of compositions embodied herein, over a period of time in response to a candidate therapeutic agent.

[0164] In another preferred embodiment, soluble and/or membrane-bound forms of compositions embodied herein, e.g. proteins, mutants or biologically active portions thereof, can be used in the assays for screening candidate agents. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, TRITON™ X-100, TRITON™ X-114, THESIT™, Isotridecypoly(ethylene glycol ether)n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.

[0165] Cell-free assays can also be used and involve preparing a reaction mixture which includes BAG3 molecules (nucleic acids or peptides) comprising a bioluminescent moiety and the test compound under conditions and time periods to allow the measurement of the translational and/or transcriptional activity over time, and concentrations of test agents.

[0166] In other embodiments, a candidate agent is an antisense oligonucleotide. In embodiments, BAG3 expression (e.g., protein) in a sample (e.g., cells or tissues in vivo or in vitro) treated using an antisense oligonucleotide of the invention is evaluated by comparison with BAG3 expression in a control sample. For example, the translation of the BAG3 is monitored by the signal emitted by the detectable moiety and compared with that in a mock-treated or untreated sample. Alternatively, comparison with a sample treated with a control antisense oligonucleotide (e.g., one having an altered or different sequence) can be made depending on the information desired. In another embodiment, a difference in the translational and/or transcriptional activity in a treated vs. an untreated sample can be compared with the difference in expression of a different nucleic acid (including any standard deemed appropriate by the researcher, e.g., a housekeeping gene) in a treated sample vs. an untreated sample.

[0167] Observed differences can be expressed as desired, e.g., in the form of a ratio or fraction, for use in a comparison with control. In some embodiments, the level of BAG3 protein, in a sample treated with an antisense oligonucleotide, is increased or decreased by about 1.25-fold to about 10-fold or more relative to an untreated sample or a sample treated with a control nucleic acid. Preferably, the level or amount of BAG3 is increased. In embodiments, the level of BAG3 protein is increased or decreased by at least about 1.25-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, or at least about 10-fold or

more. In embodiments, it is preferable that the level or amount of BAG3 is increased.

[0168] Microarrays: Identification of a nucleic acid sequence capable of binding to a target molecule can be achieved by immobilizing a library of nucleic acids onto the substrate surface so that each unique nucleic acid is located at a defined position to form an array. In general, the immobilized library of nucleic acids are exposed to a biomolecule or candidate agent under conditions which favored binding of the biomolecule to the nucleic acids. The nucleic acid array would then be analyzed by the methods embodied herein to determine which nucleic acid sequences bound to the biomolecule. Preferably the biomolecules would carry a pre-determined label for use in detection of the location of the bound nucleic acids.

[0169] An assay using an immobilized array of BAG3 nucleic acid sequences may be used for determining the sequence of an unknown nucleic acid; single nucleotide polymorphism (SNP) analysis; analysis of BAG3 gene expression patterns from a particular species, tissue, cell type, etc.; gene identification; etc.

[0170] In further embodiments, oligonucleotides or longer fragments derived from any of the BAG3 polynucleotide sequences, may be used as targets in a microarray. The microarray can be used to monitor the identity and/or expression level of large numbers of genes and gene transcripts simultaneously to identify genes with which target genes or its product interacts and/or to assess the efficacy of candidate therapeutic agents in regulating expression products of genes that mediate, for example, neurological disorders. This information may be used to determine gene function, and to develop and monitor the activities of therapeutic agents.

[0171] Microarrays may be prepared, used, and analyzed using methods known in the art (see, e.g., Brennan et al., 1995, U.S. Pat. No. 5,474,796; Schena et al., 1996, *Proc. Natl. Acad. Sci.* U.S.A. 93: 10614-10619; Baldeschweiler et al., 1995, PCT application WO95/251116; Shalon, et al., 1995, PCT application W095/35505; Heller et al., 1997, *Proc. Natl. Acad. Sci.* U.S.A. 94: 2150-2155; and Heller et al., 1997, U.S. Pat. No. 5,605,662). In other embodiments, a microarray comprises BAG3 peptides, or other desired molecules which can be assayed to identify a candidate agent.

[0172] In another preferred embodiment a method for screening candidate agents for the treatment or prevention of a cardiac disease or disorder comprises contacting a sample with a candidate therapeutic agent and measuring the effects the agent has on a target. For example, the agent may regulate BAG3 expression and the agent can then be further studied for any possible therapeutic effects (increase or decrease parameter being monitored e.g. expression). An abnormal expression state may be caused by pathology such as heart failure, disease, cancer, genetic defects and/or a toxin.

[0173] Antibodies. Useful diagnostic assays can include one or more antibodies that specifically bind BAG3. In some embodiments, the antibody specifically binds a mutant BAG3, for example, the BAG3 polypeptide disclosed herein having the 10 amino acid deletion as shown in FIG. 2. We use the term antibody to broadly refer to immunoglobulin-based binding molecules, and the term encompasses conventional antibodies (e.g., the tetrameric antibodies of the G class (e.g., an IgG1)), fragments thereof that retain the ability to bind their intended target (e.g., an Fab' fragment), and single chain antibodies (scFvs). The antibody may be polyclonal or monoclonal and may be produced by human, mouse, rabbit, sheep or goat cells, or by hybridomas derived from these cells. The antibody can be humanized, chimeric, or bi-specific.

[0174] The antibodies can assume various configurations and encompass proteins consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Any one of a variety of antibody structures can be used, including the intact antibody, antibody multimers, or antibody fragments or other variants thereof that include functional, antigen-binding regions of the antibody. We may use the term "immunoglobulin" synonymously with "antibody." The antibodies may be monoclonal or polyclonal in origin. Regardless of the source of the antibody, suitable antibodies include intact antibodies, for example, IgG tetramers having two heavy (H) chains and two light (L) chains, single chain antibodies, chimeric antibodies, humanized antibodies, complementary determining region (CDR)-grafted antibodies as well as antibody fragments, e.g., Fab, Fab', F(ab')2, scFv, Fv, and recombinant antibodies derived from such fragments, e.g., camelbodies, microantibodies, diabodies and bispecific antibodies.

[0175] An intact antibody is one that comprises an antigen-binding variable region (V_H and V_L) as well as a light chain constant domain (C_L) and heavy chain constant domains, C_{H1}, C_{H2} and C_{H3}. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variants thereof As is well known in the art, the V_H and V_L regions are further subdivided into regions of hypervariability, termed "complementarity determining regions" (CDRs), interspersed with the more conserved framework regions (FRs).

[0176] An anti-BAG3 antibody can be from any class of immunoglobulin, for example, IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof (e.g., IgG_1, IgG_2, IgG_3, and IgG_4)), and the light chains of the immunoglobulin may be of types kappa or lambda. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA_1 and IgA_2), gamma (IgG_1, IgG_2, IgG_3, IgG_4), delta, epsilon, and mu constant region genes, as well as the many immunoglobulin variable region genes.

[0177] The term "antigen-binding portion" of an immunoglobulin or antibody refers generally to a portion of an immunoglobulin that specifically binds to a target, in this case, an epitope comprising amino acid residues on a BAG3 polypeptide. An antigen-binding portion of an immunoglobulin is therefore a molecule in which one or more immunoglobulin chains are not full length, but which specifically binds to a cellular target. Examples of antigen-binding portions or fragments include: (i) an Fab fragment, a monovalent fragment consisting of the VLC, VHC, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fv fragment consisting of the VLC and VHC domains of a single arm of an antibody, and (v) an isolated CDR having sufficient framework to specifically bind, e.g., an antigen binding portion of a variable region. An antigen-binding portion of a light chain variable region and an antigen binding portion of a heavy chain variable region, e.g., the two domains of the Fv fragment, VLC and VHC, can be joined, using recombinant methods, by a

synthetic linker that enables them to be made as a single protein chain in which the VLC and VHC regions pair to form monovalent molecules (known as single chain Fv (scFv). Such scFvs can be a target agent of the present invention and are encompassed by the term "antigen-binding portion" of an antibody.

[0178] An "Fv" fragment is the minimum antibody fragment that contains a complete antigen-recognition and binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, con-covalent association. It is in this configuration that three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. While six hypervariable regions confer antigen-binding specificity, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. To improve stability, the VH-VL domains may be connected by a flexible peptide linker such as $(Gly_4Ser)_3$ to form a single chain Fv or scFV antibody fragment or may be engineered to form a disulfide bond by introducing two cysteine residues in the framework regions to yield a disulfide stabilized Fv (dsFv). Fragments of antibodies are suitable for use in the methods provided so long as they retain the desired specificity of the full-length antibody and/or sufficient specificity to specifically bind to a BAG3 polypeptide.

[0179] The compositions of the present invention include antibodies that (1) exhibit a threshold level of binding activity; and/or (2) do not significantly cross-react with known related polypeptide molecules. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, *Ann. NY Acad. Sci.* 51:660-672 (1949)).

[0180] In some embodiments, the anti-BAG3 antibodies can bind to their target epitopes or mimetic decoys at least 1.5-fold, 2-fold, 5-fold 10-fold, 100-fold, 10^3-fold, 10^4-fold, 10^5-fold, 10^6-fold or greater for the target anti-BAG3 than to other proteins predicted to have some homology to BAG3.

[0181] In some embodiments the anti- BAG3 antibodies bind with high affinity of 10^{-4}M or less, 10^{-7}M or less, 10^{-9}M or less or with subnanomolar affinity (0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 nM or even less). In some embodiments the binding affinity of the anti-BAG3 antibodies for their respective targets is at least 1×10^6 Ka. In some embodiments the binding affinity of the anti-BAG3 antibodies for BAG3 is at least 5×10^6 Ka, at least 1×10^7 Ka, at least 2×10^7 Ka, at least 1×10^8 Ka, or greater. Antibodies may also be described or specified in terms of their binding affinity to BAG3. In some embodiments binding affinities include those with a Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-3}M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7}M, 10^{-7} M, 5×10^{-8}M, 10^{-8}M, 5×10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11}M, 5×10^{-12}M, 10^{-12}M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14}M, 10^{-14}M, 5×10^{-15}M, or 10^{-15}M, or less.

[0182] In some embodiments, the antibodies do not bind to known related polypeptide molecules; for example, they bind BAG3, but not known related polypeptides. In some embodiments, the antibodies specifically bind to a mutant BAG3 polypeptide, for example a BAG3 polypeptide having the ten base pair deletion as shown in FIG. 2, but not to a wild type BAG3 polypeptide. Antibodies may be screened against known related polypeptides to isolate an antibody population that specifically binds BAG3.

[0183] The diagnostic assays of the invention can include concurrent immunoelectrophoresis, radioimmunoassay (RIA), radioimmunoprecipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. The anti-BAG3 antibodies can include a tag, which may also be referred to as a reporter or marker (e.g., a detectable marker). A detectable marker can be any molecule that is covalently linked to the anti-BAG3 antibody or a biologically active fragment thereof that allows for qualitative and/or quantitative assessment of the expression or activity of the tagged peptide. The activity can include a biological activity, a physico-chemical activity, or a combination thereof. Both the form and position of the detectable marker can vary, as long as the labeled antibody retains biological activity. Many different markers can be used, and the choice of a particular marker will depend upon the desired application. Labeled anti-BAG3 antibodies can be used, for example, for assessing the levels of BAG3 or a mutant BAG3 in a biological sample, e.g., urine, saliva, cerebrospinal fluid, blood or a biopsy sample or for evaluation the clinical response to a cardiovascular therapeutic, for example, the BAG3 constructs described above.

[0184] Exemplary detectable labels include a radiopaque or contrast agents such as barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, and thallous chloride. Alternatively or in addition, the detectable label can be a fluorescent label, for example, fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine; a chemiluminescent compound selected from the group consisting of luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester; a liposome or dextran; or a bioluminescent compound such as luciferin, luciferase and aequorin.

[0185] Suitable markers include, for example, enzymes, photo-affinity ligands, radioisotopes, and fluorescent or chemiluminescent compounds. Methods of introducing detectable markers into peptides are well known in the art. Markers can be added during synthesis or post-synthetically. Recombinant anti-BAG3 antibodies or biologically active variants thereof can also be labeled by the addition of labeled precursors (e.g., radiolabeled amino acids) to the culture medium in which the transformed cells are grown. In some embodiments, analogues or variants of peptides can be used in order to facilitate incorporation of detectable markers. For example, any N-terminal phenylalanine residue can be replaced with a closely related aromatic amino acid, such as tyrosine, that can be easily labeled with 125I. In some embodiments, additional functional groups that support effective labeling can be added to the fragments of an anti-BAG3 antibody or biologically active variant thereof. For example, a 3-tributyltinbenzoyl group can be added to the N-terminus of the native structure; subsequent displacement of the tributyltin group with 125I will generate a radiolabeled iodobenzoyl group.

[0186] Any art-known method can be used for detecting such labels, for example, positron-emission tomography (PET), SPECT imaging, magnetic resonance imaging, X-ray, or is detectable by ultrasound.

[0187] In other preferred embodiments, a method of treating a patient having a cardiac disease or disorder, wherein the patient has decreased BAG3 levels as compared to a baseline level, comprising administering a pharmaceutical composition comprising a therapeutically effective amount of at least one BAG3 inducing agent wherein the agent increases expression of the BAG3 molecule.

[0188] In other embodiments, a method of preventing or treating a subject at risk of or suffering from a cardiac disease or disorder comprising: administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide. In preferred embodiments the cardiac disease and/or disorder is heart failure.

[0189] In other embodiments, a method of treating heart failure in a patient, comprising administering a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide.

[0190] In other embodiments, a method of preventing or treating a cardiac disease or disorder in a subject, comprising administering a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide.

[0191] In yet other embodiments, a method of preventing or treating a cardiac disease or disorder in a subject, comprising administering a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide and one or more therapeutic agents prescribed by the medical caregiver. In embodiments, the at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide and one or more therapeutic agents prescribed by the medical caregiver are administered consecutively or at the same time.

Diagnostics, Therapeutics, Kits

[0192] The compositions herein and compounds of the present invention can be utilized for diagnostics, therapeutics, and prophylaxis, and as research reagents and components of kits.

[0193] The compositions disclosed herein are generally and variously useful for treatment of a subject having a cardiac disease or disorder, for example, heart failure or dilated cardiomyopathy. We may refer to a subject, patient, or individual interchangeably. A subject is effectively treated whenever a clinically beneficial result ensues. This may mean, for example, a complete resolution of the symptoms of a disease, a decrease in the severity of the symptoms of the disease, or a slowing of the disease's progression. These methods can further include the steps of a) identifying a subject (e.g., a patient and, more specifically, a human patient) who has a cardiac disease or disorder; and b) providing to the subject with a composition comprising a nucleic acid encoding a BAG3 polypeptide. The nucleic acid encoding the BAG3 polypeptide can be inserted into a vector, for example, an AAV vector, which is administered to the subject. A subject can be identified using standard clinical tests relating to cardiac function, for example. An amount of such a composition provided to the subject that results in a complete resolution of the symptoms of the infection, a decrease in the severity of the symptoms of the infection, or a slowing of the infection's progression is considered a therapeutically effective amount. The present methods may also include a monitoring step to help optimize dosing and scheduling as well as predict outcome. In some methods of the present invention, one can first determine whether a patient has decreased levels of BAG3 and then make a determination as to whether or not to treat the patient with one or more of the compositions described herein. BAG3 levels can be assayed using, for example, an anti-BAG3 antibody, and then compared to a reference level to determine whether the patient has elevated levels of BAG3. Monitoring can also be used to rapidly distinguish responsive patients from nonresponsive patients.

[0194] Cardiovascular disorders amenable to the therapeutic, and/or prognostic methods of the invention can be disorders that are responsive to the modulation of BAG3. While we believe we understand certain events that occur in the course of treatment, the compositions of the present invention are not limited to those that work by affecting any particular cellular mechanism. Any form of cardiovascular disorder which is associated with misregulation of BAG3 is within the scope of the invention.

[0195] The methods of the invention can be expressed in terms of the preparation of a medicament. Accordingly, the invention encompasses the use of the agents and compositions described herein in the preparation of a medicament. The compounds described herein are useful in therapeutic compositions and regimens or for the manufacture of a medicament for use in treatment of diseases or conditions as described herein (e.g., a cardiovascular disorder disclosed herein).

[0196] Any composition described herein can be administered to any part of the host's body for subsequent delivery to a target cell. A composition can be delivered to, without limitation, the brain, the cerebrospinal fluid, joints, nasal mucosa, blood, lungs, intestines, muscle tissues, skin, or the peritoneal cavity of a mammal. In terms of routes of delivery, a composition can be administered by intravenous, intracranial, intraperitoneal, intramuscular, subcutaneous, intramuscular, intrarectal, intravaginal, intrathecal, intratracheal, intradermal, or transdermal injection, by oral or nasal administration, or by gradual perfusion over time. In a further example, an aerosol preparation of a composition can be given to a host by inhalation.

[0197] The dosage required will depend on the route of administration, the nature of the formulation, the nature of the patient's illness, the patient's size, weight, surface area, age, and sex, other drugs being administered, and the judgment of the attending clinicians. Suitable dosages are in the range of 0.01-1,000 mg/kg. Wide variations in the needed dosage are to be expected in view of the variety of cellular targets and the differing efficiencies of various routes of administration. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art. Administrations can be single or multiple (e.g., 2- or 3-, 4-, 6-, 8-, 10-, 20-, 50-, 100-, 150-, or more fold). Encapsulation of the compounds in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery.

[0198] The duration of treatment with any composition provided herein can be any length of time from as short as one day to as long as the life span of the host (e.g., many years). For example, a compound can be administered once a week (for, for example, 4 weeks to many months or years); once a month (for, for example, three to twelve months or for many years); or once a year for a period of 5 years, ten years, or longer. It is also noted that the frequency of treatment can be variable. For example, the present compounds can be administered once (or twice, three times, etc.) daily, weekly, monthly, or yearly.

[0199] An effective amount of any composition provided herein can be administered to an individual in need of treatment. The term "effective" as used herein refers to any amount that induces a desired response while not inducing significant toxicity in the patient. Such an amount can be determined by assessing a patient's response after administration of a known amount of a particular composition. In addition, the level of toxicity, if any, can be determined by assessing a patient's clinical symptoms before and after administering a known amount of a particular composition. It is noted that the effective amount of a particular composition administered to a patient can be adjusted according to a desired outcome as well as the patient's response and level of toxicity. Significant toxicity can vary for each particular patient and depends on multiple factors including, without limitation, the patient's disease state, age, and tolerance to side effects.

[0200] Any method known to those in the art can be used to determine if a particular response is induced. Clinical methods that can assess the degree of a particular disease state can be used to determine if a response is induced. The particular methods used to evaluate a response will depend upon the nature of the patient's disorder, the patient's age, and sex, other drugs being administered, and the judgment of the attending clinician.

[0201] Concurrent administration of two or more therapeutic agents does not require that the agents be administered at the same time or by the same route, as long as there is an overlap in the time period during which the agents are exerting their therapeutic effect. Simultaneous or sequential administration is contemplated, as is administration on different days or weeks. The compositions may also be administered with another standard therapeutic agent for treatment of cardiovascular disease.

[0202] In another preferred embodiment, the agents modulate the expression of Bcl-2 associated anthanogene-3 (BAG3) in patients suffering from or at risk of developing diseases or disorders associated with molecules or pathways associated with BAG3. Examples of such diseases or disorders associated comprise: cardiac diseases or disorders, skeletal muscle diseases or disorders, multiple sclerosis, senile plaques, cerebral amyloid angiopathy, atherosclerosis, glioblastoma, amyloid deposition, neurodegenerative diseases, neurofibrillary tangles, dementia, choriocarcinoma, astrocytoma, amyloidosis, hyperlipidemia, neurodegeneration, neoplastic transformation, prostate cancer, atherosclerotic plaque, obstruction, AIDS, metastasis, myocardial infarction, pulmonary fibrosis, necrosis, shock, melanoma, colorectal carcinoma, genetic susceptibility, psoriasis, cancer, inflammation, glioma, carcinoma, breast cancer, neuropathology, tumors, prostate carcinoma, vascular diseases, cell damage, brain tumors, Non-small cell lung carcinomas (NSCLCs), hypercholesterolemia. Examples of skeletal muscle diseases include, primary (genetic) diseases of muscle (e.g., muscular dystrophies and congenital myopathies, metabolic myopathies); acquired diseases (e.g. myositis, toxic myopathy); secondary diseases of muscle (e.g. neurogenic atrophy, atrophy from chronic pulmonary, heart, kidney disease, HIV/AIDs, cancer, sarcopenia and the like.

[0203] Kits: The present invention further provides systems and kits (e.g., commercial therapeutic, diagnostic, or research products, reaction mixtures, etc.) that contain one or more or all components sufficient, necessary, or useful to practice any of the methods described herein. These systems and kits may include buffers, detection/imaging components, positive/negative control reagents, instructions, software, hardware, packaging, or other desired components.

[0204] The kits provide useful tools for screening test compounds capable of modulating the effects of a compound on a target molecule. The kits can be packaged in any suitable manner to aid research, clinical, and testing labs, typically with the various parts, in a suitable container along with instructions for use.

[0205] In certain embodiments, the kits may further comprise lipids and/or solvents. In certain embodiments, the kits may further comprise buffers and reagents needed for the procedure, and instructions for carrying out the assay. In certain embodiments, the kits may further comprise, where necessary, agents for reducing the background interference in a test, positive and negative control reagents, apparatus for conducting a test, and the like.

[0206] Also provided are kits for determining whether a subject has a mutation in a BAG3 polypeptide, for example, the 10 base pair deletion disclosed herein, to diagnose patients having cardiovascular disease or a predisposition to developing cardiovascular disease. The kits can also be utilized to monitor the efficiency of agents used for treatment of cardiovascular disease.

Administration of Compositions

[0207] The agents identified by the methods embodied herein can be formulated and compositions of the present invention may be administered in conjunction with one or more additional active ingredients, pharmaceutical compositions, or other compounds. The therapeutic agents of the present invention may be administered to an animal, preferably a mammal, most preferably a human.

[0208] In some embodiments, a pharmaceutical composition comprises a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide is administered as part of the treatment.

[0209] In some embodiments, a pharmaceutical composition comprises a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide and one or more therapeutic agents prescribed by the medical caregiver.

[0210] In other embodiments, a pharmaceutical composition comprises at least one or more candidate therapeutic agents embodied herein.

[0211] The pharmaceutical formulations may be for administration by oral (solid or liquid), parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), intracardial, transdermal (either passively or using ionophoresis or electroporation), transmucosal and systemic (nasal, vaginal, rectal, or sublingual), or inhalation

routes of administration, or using bioerodible inserts and can be formulated in dosage forms appropriate for each route of administration.

[0212] The agents may be formulated in pharmaceutically acceptable carriers or diluents such as physiological saline or a buffered salt solution. Suitable carriers and diluents can be selected on the basis of mode and route of administration and standard pharmaceutical practice. A description of exemplary pharmaceutically acceptable carriers and diluents, as well as pharmaceutical formulations, can be found in Remington's Pharmaceutical Sciences, a standard text in this field, and in USP/NF. Other substances may be added to the compositions to stabilize and/or preserve the compositions.

[0213] The compositions of the invention may be administered to animals by any conventional technique. The compositions may be administered directly to a target site by, for example, surgical delivery to an internal or external target site, or by catheter to a site accessible by a blood vessel. Other methods of delivery, e.g., liposomal delivery or diffusion from a device impregnated with the composition, are known in the art. The compositions may be administered in a single bolus, multiple injections, or by continuous infusion (e.g., intravenously). For parenteral administration, the compositions are preferably formulated in a sterilized pyrogen-free form.

[0214] The compounds identified by this invention may also be administered orally to the patient, in a manner such that the concentration of drug is sufficient to inhibit bone resorption or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the condition of the patient. Preferably the oral dose would be about 0.5 to about 20 mg/kg.

[0215] An intravenous infusion of the compound in 5% dextrose in water or normal saline, or a similar formulation with suitable excipients, is most effective, although an intramuscular bolus injection is also useful. Typically, the parenteral dose will be about 0.01 to about 100 mg/kg; preferably between 0.1 and 20 mg/kg, in a manner to maintain the concentration of drug in the plasma at a concentration effective to increase BAG3 expression. The compounds may be administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about 400 mg/kg/day. The precise amount of an inventive compound which is therapeutically effective, and the route by which such compound is best administered, is readily determined by one of ordinary skill in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect. Prodrugs of compounds of the present invention may be prepared by any suitable method.

[0216] No unacceptable toxicological effects are expected when compounds, derivatives, salts, compositions etc., of the present invention are administered in accordance with the present invention. The compounds of this invention, which may have good bioavailability, may be tested in one of several biological assays to determine the concentration of a compound which is required to have a given pharmacological effect.

[0217] In another preferred embodiment, there is provided a pharmaceutical or veterinary composition comprising one or more identified compounds and a pharmaceutically or veterinarily acceptable carrier. Other active materials may also be present, as may be considered appropriate or advisable for the disease or condition being treated or prevented.

[0218] The carrier, or, if more than one be present, each of the carriers, must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient.

[0219] The compounds identified by the methods herein would be suitable for use in a variety of drug delivery systems described above. Additionally, in order to enhance the in vivo serum half-life of the administered compound, the compounds may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional techniques may be employed which provide an extended serum half-life of the compounds. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka, et al., U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028 each of which is incorporated herein by reference. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody. The liposomes will be targeted to and taken up selectively by the organ.

[0220] The formulations include those suitable for rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration, but preferably the formulation is an orally administered formulation. The formulations may conveniently be presented in unit dosage form, e.g. tablets and sustained release capsules, and may be prepared by any methods well known in the art of pharmacy.

[0221] Such methods include the step of bringing into association the above defined active agent with the carrier. In general, the formulations are prepared by uniformly and intimately bringing into association the active agent with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

[0222] The compound identified using these methods can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the compound is combined in admixture with a pharmaceutically acceptable carrier vehicle. Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, (ICI Americas Inc., Bridgewater, N.J.), PLURONICS™, (BASF Corporation, Mount Olive, N.J.) or PEG.

[0223] The formulations to be used for in vivo administration must be sterile and pyrogen free. This is readily

accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution.

[0224] Dosages and desired drug concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" In Toxicokinetics and New Drug Development, Yacobi et al., Eds., Pergamon Press, New York 1989, pp. 42-96.

[0225] Formulations for oral administration in the present invention may be presented as: discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active agent; as a powder or granules; as a solution or a suspension of the active agent in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water in oil liquid emulsion; or as a bolus etc.

[0226] For compositions for oral administration (e.g. tablets and capsules), the term "acceptable carrier" includes vehicles such as common excipients e.g. binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone (Povidone), methylcellulose, ethylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sucrose and starch; fillers and carriers, for example corn starch, gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid; and lubricants such as magnesium stearate, sodium stearate and other metallic stearates, glycerol stearate stearic acid, silicone fluid, talc waxes, oils and colloidal silica. Flavoring agents such as peppermint, oil of wintergreen, cherry flavoring and the like can also be used. It may be desirable to add a coloring agent to make the dosage form readily identifiable. Tablets may also be coated by methods well known in the art.

[0227] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active agent in a free flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active agent.

[0228] Other formulations suitable for oral administration include lozenges comprising the active agent in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active agent in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active agent in a suitable liquid carrier.

[0229] Parenteral formulations will generally be sterile.

[0230] Dose: An effective dose of a composition of the presently disclosed subject matter is administered to a subject in need thereof. A "therapeutically effective amount" or a "therapeutic amount" is an amount of a therapeutic composition sufficient to produce a measurable response (e.g., a biologically or clinically relevant response in a subject being treated). The response can be measured in many ways, as discussed above, e.g. cytokine profiles, cell types, cell surface molecules, etc. Actual dosage levels of active ingredients in the compositions of the presently disclosed subject matter can be varied so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular subject. The selected dosage level will depend upon the activity of the therapeutic composition, the route of administration, combination with other drugs or treatments, the severity of the condition being treated, and the condition and prior medical history of the subject being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. The potency of a composition can vary, and therefore a "treatment effective amount" can vary. However, using the assay methods described herein, one skilled in the art can readily assess the potency and efficacy of a candidate compound of the presently disclosed subject matter and adjust the therapeutic regimen accordingly.

[0231] The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modifications and improvements within the spirit and scope of the invention.

[0232] All documents mentioned herein are incorporated herein by reference. All publications and patent documents cited in this application are incorporated by reference for all purposes to the same extent as if each individual publication or patent document were so individually denoted. By their citation of various references in this document, Applicants do not admit any particular reference is "prior art" to their invention.

EXAMPLES

[0233] While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and not limitation. The following non-limiting examples are illustrative of the invention.

Example 1

Changes in BAG3 Protein Levels are Associated with Both Familial and Non-Familial Dilated Cardiomyopathy

[0234] Mutations in Bcl-2 associated anthanogene-3 (BAG3), a 575 amino acid anti-apoptotic protein that serves as a co-chaperone of the heat shock proteins (HSPs), has been associated with FDC (Selcen D, et al., *Annals of Neurology*. 2009;65:83-89; Odgerel Z, et al., *Neuromuscular disorders : NMD*. 2010; 20:438-442; Lee H C, et al., *Clinical Genetics*. 2012; 81:394-398). For example, Norton et al. recently identified a deletion of BAG3 exon 4 as a rare variant causative of FDC in a family without neuropathy or peripheral muscle weakness (Norton N, et al., *American Journal of Human Genetics*. 2011; 88:273-282). Subsequent sequencing of BAG3 in subjects diagnosed with IDC identified four additional mutations that segregated with all relatives affected by the disease. A genome-wide association study conducted in patients with HF secondary to IDC implicated a non-synonymous single nucleotide polymor-

phism (SNP) (c.757T>C, [p. Cys151Arg]) located within the BAG3 gene as contributing to sporadic dilated cardiomyopathy (Villard E, et al., *European Heart Journal.* 2011; 32:1065-1076).

[0235] In the present study, a novel BAG3 mutation was identified in a family with adult-onset FDC. Furthermore, it is reported herein, for the first time, that BAG3 protein levels are significantly decreased in unrelated patients with non-familial IDC evidencing that altered levels of BAG3 protein may participate in the progression of HF.

[0236] Materials and Methods

[0237] Materials: A family with adult-onset familial dilated cardiomyopathy was identified. After obtaining informed consent, participating family members underwent a physical examination by a heart failure cardiologist and blood was collected for subsequent DNA analysis. DNA was extracted using a DNA extraction kit (Qiagen, Valencia Calif.) and stored at −70° C. Whenever possible, electrocardiograms were obtained from affected family members who had not undergone heart transplantation. Family members who had not had a recent echocardiogram underwent a transthoracic echocardiogram using a SonoHeart Elite (SonoSite Inc, Bothell, Washington, USA) portable echocardiographic system. Medical records were obtained from one individual who had died. Affection status was determined on the basis of consensus guidelines (Mestroni L, et al., *European Heart Journal.* 1999; 20:93-102). Participating family members provided written informed consent prior to evaluation and the protocols were approved by the Internal Review Boards of Thomas Jefferson University and of the University of Colorado.

[0238] Methods: Human heart tissue was obtained from 9 subjects unrelated to the study family with end-stage heart failure undergoing heart transplant at Temple University Hospital (6 male, 3 female, mean age 47.6±5.7 years), from one affected family member at the time of heart transplantation at the University of Colorado and from 7 organ donors (1 male, 6 female, mean age 59.3 ±3.7 years) whose hearts were unsuitable for donation owing to blood type, age or size incompatibility. All of the patients undergoing transplantation had severe left ventricular dysfunction and cardiac dilation with a mean left ventricular ejection fraction (LVEF) of 12.8±1.4%. Two of the transplant recipients had HF secondary to ischemic cardiomyopathy and the remainder had non-ischemic IDC. Four of the transplant recipients were receiving dobutamine alone, 5 were receiving milrinone alone and one was receiving both milrinone and dobutamine at the time of the transplantation. Echocardiography was performed on all of the organ donors prior to organ donation and all had normal left ventricular function by echocardiography with a mean LVEF of 57.5±1.6%. Tissue aliquots were removed from the left ventricular free wall, rapidly frozen in liquid nitrogen and stored at −70° C. as described previously (Bristow M R, et al., *The Journal of Clinical Investigation.* 1993; 92:2737-2745). The Institutional Review Boards of the University of Colorado and Temple University approved the tissue study and consent was obtained for all subjects.

[0239] Exome Sequencing and Bioinformatics: DNA from 5 affected family members and 1 unaffected family member was selected for exome sequencing with a target depth of >100×. Exome enrichment was performed using the Agilent SureSelect Human Exon 51Mb kit (Agilent, Santa Clara, Calif.). Paired-end 100 nucleotide exome sequencing was performed using an Illumina HiSeq 2000 platform (San Diego, Calif.). Sequence reads passing Illumina chastity filter, were subjected to a quality filter step, trimmed and retained if the trimmed reads for each pair exceeded 50 nucleotides. Paired reads were then mapped to the reference human genome sequence (hg19) with gSNAP (Wu T D, et al., *Bioinformatics.* 2010; 26:873-881). Sequence calls for variants (single-nucleotide polymorphisms [SNPs)] insertions and deletions [indels]) were performed using the Broad's Genome Analysis Toolkit (McKenna A, et al., *Genome Research.* 2010; 20:1297-1303).

[0240] After variant detection, the program Annotate Variation (ANNOVAR) was used to classify variants (e.g., exonic, intronic, synonymous, non-synonymous, splice variant, stop gain, stop loss, insertion, or deletion) and to cross reference all the variants across various genetic variation databases (e.g., dbSNP, 1000 genomes database, AVSIFT) to isolate rare variants (variants with mean allele frequencies of ≤1% not found in dbSNP, 1000 genomes database, aVSIFT) (Wang K, et al., *Nucleic Acids Research.* 2010; 38:e164). Only non-synonymous changes (SNPs and in-dels), those that cause an alternate splice site, and/or an aberrant stop codon, were considered for further analysis. For non-synonymous changes, all insertion and deletion variants were considered damaging, whereas SNP variants were cross-referenced to the dbNSFP database to determine whether the changes to the protein structure would be considered tolerable or damaging using four algorithms (Sorting Intolerant From Tolerant (SIFT), PolyPhen2, likelihood ratio test [LRT], or MutationTaster) (Liu X, et al., *Human Mutation.* 2011; 32:894-899). Putative mutations identified were confirmed with traditional Sanger sequencing in both affected and unaffected family members (primers and conditions available upon request).

[0241] Western Blot Analysis of Human Heart Tissue: Frozen tissue was homogenized in 40 mM Tris buffer, pH 7.5 containing 150 mM NaCl, 1% NP40, 1 mM DTT, and 1 mM EDTA. The sample was then centrifuged at 10,000×g at 4° C. for 30 min and the supernatant was collected and re-suspended in 350 μM Tris buffer, pH 6.8 containing 25% beta-mercaptoethanol, 30% glycerol, 10% SDS, and 2% bromophenol blue. The protein concentration was measured using the method of Bradford and the samples were stored at −80° C. Equal amounts of protein (10 μg) were fractionated by SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membrane. Membranes were blocked in 10% nonfat dry milk/tris-buffered saline (pH 7.6) plus 0.1% Tween-20 (TBS-T) for 1 h and then incubated with polyclonal BAG3 antibody (Proteintech, Chicago, IL) in 5% nonfat dry milk with PBST for 2 hrs. Membranes were then incubated with goat-anti-rabbit 800 and goat-anti-mouse secondary antibody for 1 hr and scanned on a LI-COR Odyssey imaging system (Lincoln Nebr.). All Western blot procedures were carried out at room temperature. BAG3 signal intensity was normalized to GAPDH.

[0242] Results

[0243] Family history: The proband (FIG. 1, III-5) was a 65 year-old woman of Eastern European ancestry who was referred in June 2003 to the heart failure clinic at Thomas Jefferson University because of a family history of HF. She had first been noted to have a dilated cardiomyopathy at 45 years of age. She was largely asymptomatic while receiving a diuretic, a β-adrenergic receptor antagonist (β-blocker) and an angiotensin converting enzyme (ACE) inhibitor. Her

vital signs were within normal limits and her physical examination was notable only for a soft S3 heart sound. She had no peripheral muscle weakness and her neurologic examination was unremarkable. Her electrocardiogram revealed normal sinus rhythm with mild LV hypertrophy and non-specific ST-T wave changes. Her left ventricular ejection fraction was 20% by echocardiography. As seen in FIG. 1 and Table 1, the proband had two female siblings, one of whom (III-7) was asymptomatic with a normal physical examination; however, her ejection fraction by echocardiography was 44%. A second sister (III-9) was phenotypically normal and had a normal echocardiogram.

[0244] The proband had three children. A son underwent cardiac transplantation at the age of 20 secondary to IDC (IV-5), a second son was diagnosed with idiopathic dilated cardiomyopathy at the age of 20 but remained asymptomatic at age 32 despite an ejection fraction of 33% (IV-4). A daughter had no cardiac symptoms; however, her left ventricular ejection fraction by echocardiography was 48% and she had mild dilatation of the left ventricle and the aortic root without obvious aortic valve disease. (IV-6) Her echocardiogram met the criteria for diagnosis of a dilated cardiomyopathy. Her electrocardiogram was normal. Neurologic function was normal in all three children. The proband's affected sister (III-7) had one daughter who died of progressive heart failure secondary to IDC at the age of 22. (IV-7): two other children had normal echocardiograms. A cousin underwent cardiac transplantation because of IDC at 42 years of age after diagnosis at the age of 40 (III-1) and one of his sons also underwent cardiac transplantation for IDC at the University of Colorado at the age of 30 (IV-1). Healthy subjects were defined as "non-affected" if they had reached the age of 40 without symptoms and had a normal echocardiogram that did not meet the criteria for diagnosis of a cardiomyopathy. Ten-year follow-up of all participants demonstrated that functional capacity had remained stable in all family members.

[0245] Genetic analysis: As seen in FIG. 1, the pedigree and clinical data were compatible with autosomal dominant adult-onset familial IDC. Exome sequencing of the DNA from 5 affected (III-5, 7: IV-1,4,5) and 1 unaffected (III-9) family members had an average of 11.8±0.96 Gb of past-filter sequence reads per sample. After bioinformatics filtering a 10-nucleotide deletion in the coding portion of exon 4 of BAG3 (Ch10:del 121436332_12143641: del. 1266_1275 [NM 004281]) was noted to be present in all tested affected subjects and absent in the one healthy sister of the proband (III-9) (FIG. 2). Additional family members were tested for the BAG3 deletion by Sanger sequencing confirming appropriate co-segregation of the deletion with the phenotype among affected (III-1,5,7 and IV-1,4,5,6) and unaffected (III-9 and IV-8,9,10,11,12) individuals. This deletion was not found in existing databases and introduces a frame shift and premature stop codon after 13 amino acids that predicts truncation of BAG3 at the carboxy terminal end by 140 amino acids. Thus, the abnormal BAG3 protein is predicted to have 435 amino acids instead of 575 amino acids. In addition, the amino acid sequence distal to the deletion (K P S W R R Y R G W S R L) is predicted to be different from that found in the normal protein. Only one additional variant was found by exome sequencing and after bioinformatics filtering. The variant (rs8192669), found in the IKZF5 gene did not segregate according to the IDC phenotype in other family members. An analysis of 52 genes previously associated with monogenic IDC for rare variants (<1%) identified only non-synonymous mutations in TTN, GATAD1, MYPN, ANKRD1 and RBM20: none of these variants segregated with the disease phenotype.

[0246] BAG3 expression in failing human heart: In order to determine whether the BAG3 deletion (BAG3 del. NM 004281) found in this patient cohort resulted in a decrease in the levels of BAG3 protein, Western blot analysis was performed on cardiac muscle obtained from one affected family member (IV-1) who underwent cardiac transplantation. The level of BAG3 protein in subject IV-1 was less than half that seen in heart tissue obtained from organ donors whose heart could not be utilized for transplantation. As seen in FIGS. 3A and 3B, BAG3 levels in failing human heart from patients with end stage heart failure without known BAG3 mutations were significantly ($p=0.0002$) less than that found in non-failing control hearts. Thus it appears that decreased levels of BAG3 protein can be found both in individuals with a BAG3 mutation as well as in end-stage failing human heart.

[0247] Discussion

[0248] It is being increasingly recognized that genetic mutations can account for as many as a third of cases of IDC. Indeed, investigators have begun to refer to these cases as familial dilated cardiomyopathy (FDC). Inheritance can occur in a variety of manners with the most common pattern of inheritance being autosomal dominant. Mutations are most commonly found in genes encoding the sarcomere leading to cardiac dysfunction, disintegration of the myofiber structure and accumulation of degraded material in autophagic granules. Here, it is reported that a 10 bp deletion in the gene encoding the sarcomeric protein BAG3 segregates completely with affected individuals in a family with an autosomal dominant pattern of FDC. It is also report for the first time that BAG3 protein is substantially reduced in the hearts of unrelated patients who are undergoing heart transplantation when compared with normal hearts from transplant recipients.

[0249] BAG3 is a 575 amino acid anti-apoptotic protein that is constitutively expressed in the heart and serves as a co-chaperone of the heat shock proteins (HSPs). BAG3 binds to HSPs and regulates their ability to chaperone cytoskeletal proteins including desmin and also participate in degradation of cellular proteins through either the proteasome or autophagy pathways. BAG3 also protects cells from apoptotic death and inhibits myofibrillar degeneration in response to mechanical stress. Knockdown of BAG3 in zebrafish or in neonatal cardiomyocytes or homozygous disruption of BAG3 in mice leads to cardiac dysfunction and BAG3 levels are decreased in the skeletal muscle of spontaneously hypertensive rats.

[0250] The results of the present study in a large family with FDC are consistent with earlier reports that demon-

strated an association between mutations in BAG3 and the development of muscle pathology. Mutations in BAG3 were first shown to cause abnormal muscle function in two families with childhood-onset muscular dystrophy (Selcen D, et al., *Annals of Neurology.* 2009; 65:83-89; Odgerel Z, et al., Neuromuscular Disorders: NMD. 2010; 20:438-442) and the phenotype of IDC, diffuse myocardial fibrosis and sudden death was linked with markers in the chromosome 10q25-26 region which includes the BAG3 locus. More recent studies have demonstrated a causative relationship between BAG3 mutations and the development of FDC without peripheral muscle weakness or neurologic findings (Norton N, et al., *American Journal of Human Genetics.* 2011; 88:273-282; Villard E, et al., *European Heart Journal.* 2011; 32:1065-1076; Arimura T, et al., *Human Mutation.* 2011; 32:1481-1491).

[0251] As seen with genetic variants in other sarcomeric genes, there was substantial genetic heterogeneity within this large family. For example, one of the proband's sons had an early onset of severe disease requiring transplantation whereas a sibling with moderate disease and a middle-aged daughter with very mild disease remain asymptomatic for over a decade. Indeed, the cardiac dysfunction in the proband's daughter would have gone unrecognized had it not been for careful phenotyping as part of this study. Identification of the causative mutation in this family provides an opportunity for guideline-driven genetic testing and counseling of family members and early identification of affected individuals. The finding that use of an angiotensin converting enzyme inhibitor improved survival in a small group of patients with Duchenne muscular dystrophy suggests that early therapy in families with mutations in sarcomere genes might be beneficial; however, additional studies will be required to define the best treatment strategies.

[0252] It is reported herein, for the first time that the level of BAG3 protein is significantly reduced in the hearts of unrelated patients with end-stage HF who are undergoing heart transplant and who have no family history of heart muscle disease. This finding is interesting as it evidences that while mutations in BAG3 can be causative of disease in FDC, changes in levels of BAG3 protein may participate in the progression of disease in patients with non-familial forms of IDC. Nonetheless, these results evidence that BAG3 protein might be a new target for therapeutic intervention in HF.

Example 2

Changes in BAG3 Protein Levels in Failing Murine Hearts

[0253] Wild type c57BL/6 mice underwent trans-aortic banding (TAC) as described in Tilley et al. (*Circulation* 2014, Nov. 11; 130(20):1800-11). Eighteen weeks after TAC, left ventricular contractility was measured using a conductance catheter inserted into the left ventricle through a carotid approach as described previously. Contractility was measured during intravenous infusion of increasing doses of catecholamine. (FIG. 6B) Heart weight to body weight ratios were calculated after sacrifice. (FIG. 6A). Hearts were then frozen for subsequent measurement of BAG3 levels. Myocardial proteins were extracted as described in Example 1, separated by gel electrophoresis and probed with a murine BAG3 antibody. As shown in FIG. 6C, there was a significant decrease in BAG3 levels by Western blotting in TAC mice when compared with sham-operated controls. A representative Western blot is shown in FIG. 6D.

Example 3

Changes in BAG3 Protein Levels in Porcine Hearts Following Balloon Occlusion

[0254] Hemodynamic indices and BAG3 levels were measured in non-infarcted left ventricular myocardium from a pig 4 weeks after balloon occlusion of the left anterior descending coronary artery. As shown in 7A, 7B, 7C, and 7D, ejection fraction, fractional shortening, end-diastolic volume, and end systolic volume, respectively, were significantly altered following balloon occlusion. As shown graphically in FIG. 7E, and in the Western blot in FIG. 7F, BAG3 levels were reduced in porcine hearts following balloon occlusion.

TABLE 1

Phenotype of study subjects with and without a 10-nucleotide deletion in the BAG3 gene.

Subject	Age Eval/ Onset/Death or Transpl	Gender	EF (%)	ECG	Mutation	Comment
II-1	na/na/70+	M				Died late 70's, hx of HF
II-3	na/na/80	F				Hx of HBP and CVA
II-4	na/na/29	M				motor vehicle accident
III-1	62/40/42	M			Yes	transplant at 42
III-5	65/45/na	F	20	NS-STT changes	Yes	
III-7	67/47/na	F	44	nl	Yes	asymptomatic
III-9	68/na/na	F	58	nl	No	
IV-1	30/30/30	M			Yes	transplant at 30
IV-4	39/20/na	M	33	sinus brady, IVCD	Yes	asymptomatic
IV-5	35/20/20	M			Yes	transplant at 20
IV-6	34/34/na	F	48	nl	Yes	mild aortic root dilat, LVDD 5.8
IV-7	na/18/22	F				died-worsening HF
IV-8	38	F	nl		No	
IV-9	42	M	nl		No	
IV-10	41	F	nl		No	
IV-11	44	M	nl		No	
IV-12	45	M	nl		No	

1. A method of treating a patient suffering from, or, at risk of developing a muscle related disease or disorder comprising:

administering to the patient a therapeutically effective amount of an agent wherein the agent modulates expression or amount of BCL2-associated athanogene 3 (BAG3) molecules, proteins of peptides thereof in a target cell or tissue, as compared to a normal control.

2.-49. (canceled)

* * * * *

EXHIBIT "E"

US 20210017600A1

(19) **United States**

(12) **Patent Application Publication**

Feldman et al.

(10) **Pub. No.: US 2021/0017600 A1**

(43) **Pub. Date: Jan. 21, 2021**

(54) **BAG3 AS A TARGET FOR THERAPY OF HEART FAILURE**

(71) Applicant: **TEMPLE UNIVERSITY OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION**, Philadelphia, PA (US)

(72) Inventors: **Arthur M. Feldman**, Wynnewood, PA (US); **Douglas G. Tilley**, Bryn Mawr, PA (US); **Weizhong Zhu**, Cockeysville, MD (US); **Kamel Khalili**, Bala Cynwyd, PA (US); **Walter J. Koch**, Broomall, PA (US)

(73) Assignee: **TEMPLE UNIVERSITY OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION**, Philadelphia, PA (US)

(21) Appl. No.: **15/929,784**

(22) Filed: **May 21, 2020**

Related U.S. Application Data

(63) Continuation of application No. 15/115,807, filed on Aug. 1, 2016, filed as application No. PCT/US2015/013926 on Jan. 30, 2015.

(60) Provisional application No. 61/934,483, filed on Jan. 31, 2014.

Publication Classification

(51) **Int. Cl.**

C12Q 1/6883 (2006.01)

G01N 33/68 (2006.01)

(52) **U.S. Cl.**

CPC ***C12Q 1/6883*** (2013.01); ***G01N 33/6893*** (2013.01); ***G01N 33/6887*** (2013.01); *A61K 48/00* (2013.01); *C12Q 2600/156* (2013.01); *G01N 2800/325* (2013.01); *C12Q 2600/106* (2013.01)

(57) **ABSTRACT**

Compositions are directed to BCL2-associated athanogene 3 (BAG3) molecules and agents which modulate expression of BAG3 molecules. Pharmaceutical composition for administration to patients, for example, patients with heart failure, comprise one or more BAG3 molecules or agents which modulate expression of BAG3. Methods of treatment and identifying candidate therapeutic agents are also provided.

Specification includes a Sequence Listing.

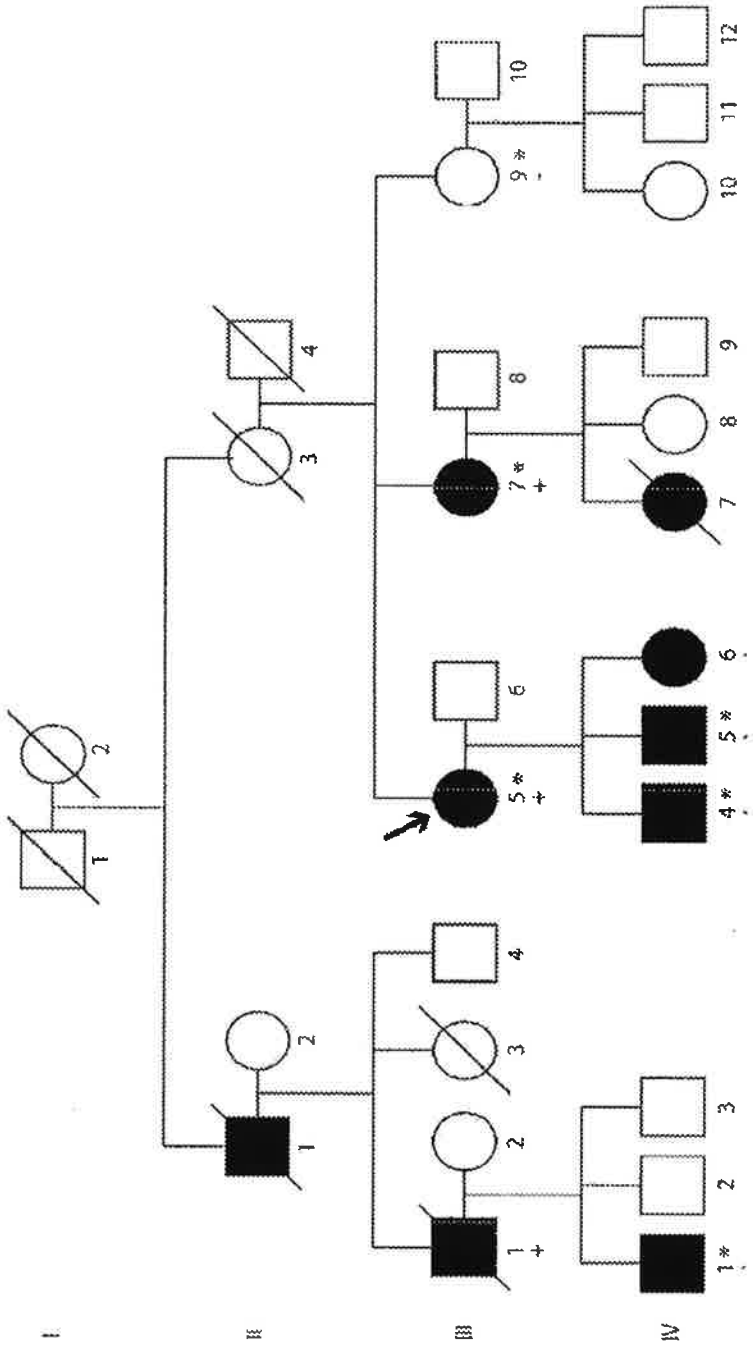


FIG. 1

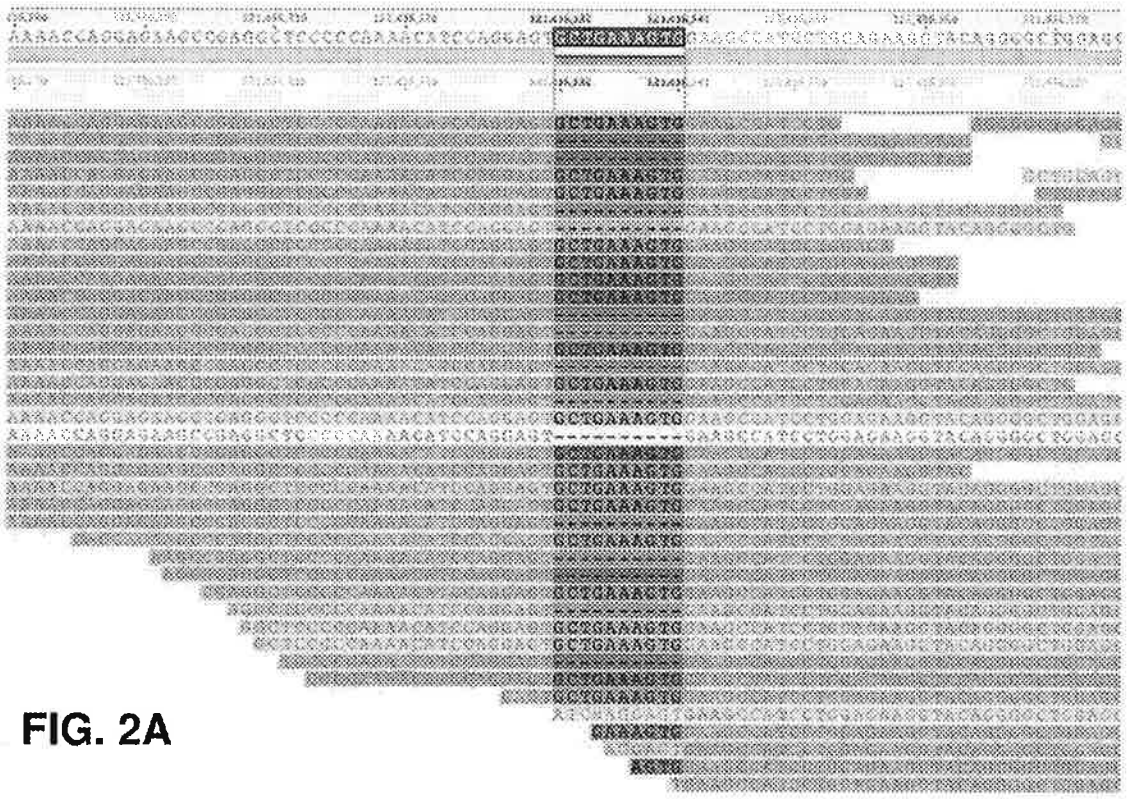


FIG. 2A

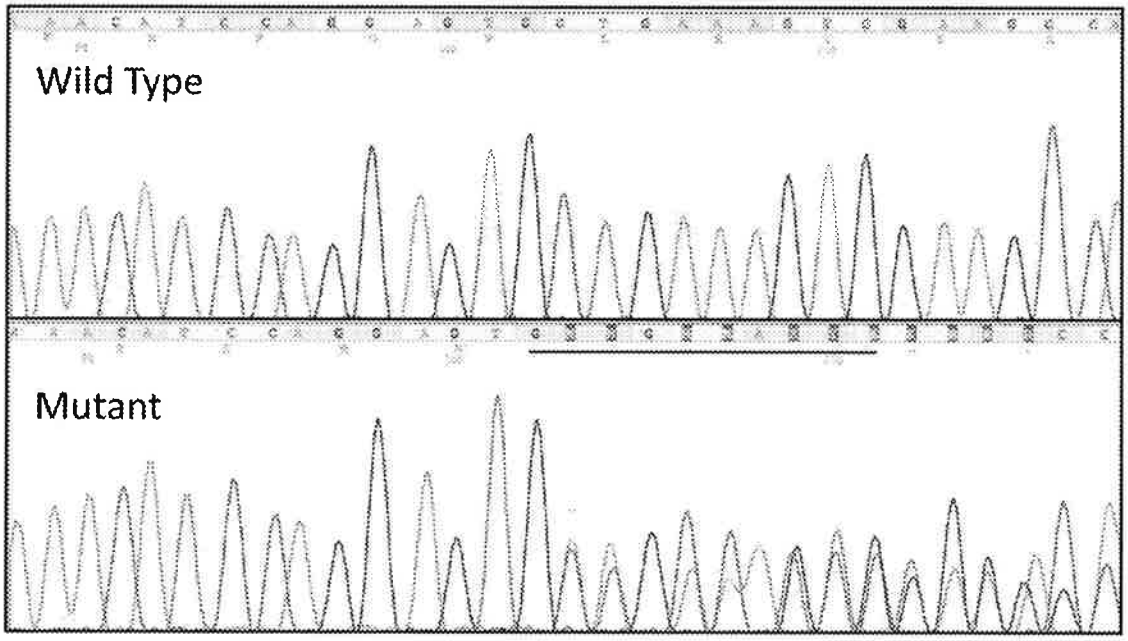


FIG. 2B

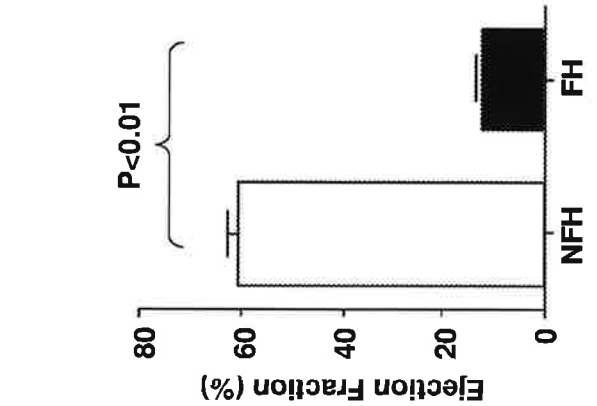

P<0.01
Ejection Fraction (%)
80
60
40
20
0
NFH
FH

FIG. 3B

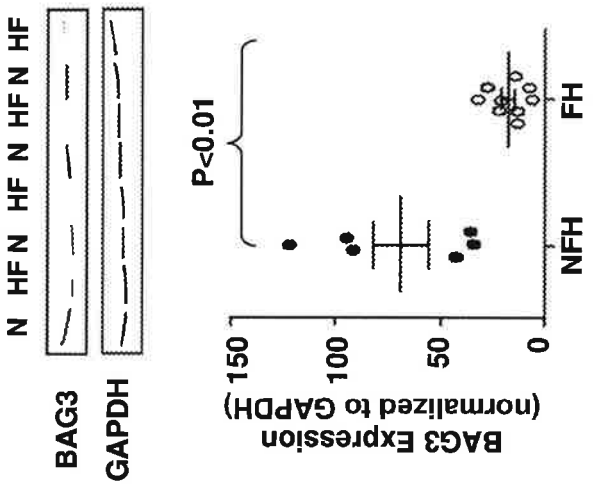

N HF N HF N HF N HF
BAG3
GAPDH
P<0.01
BAG3 Expression
(normalized to GAPDH)
150
100
50
0
NFH
FH

FIG. 3A

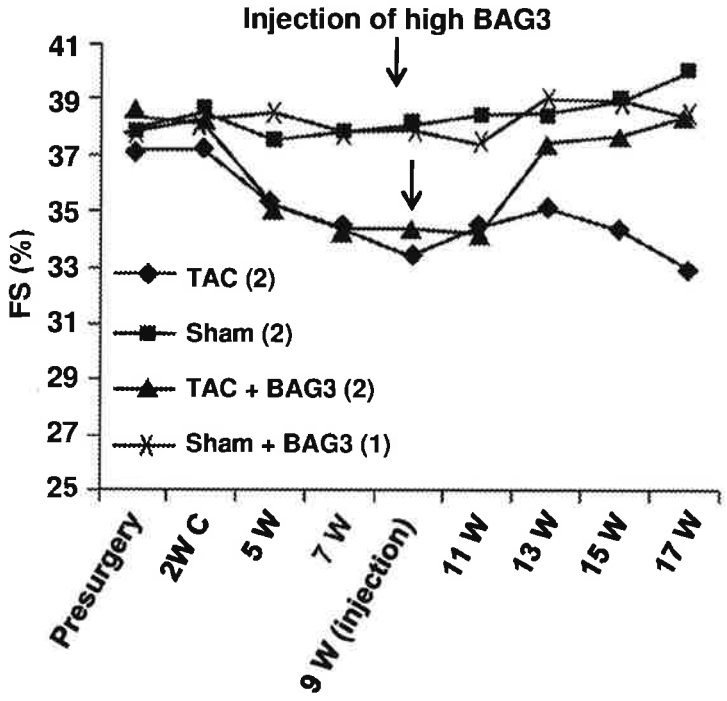

Injection of high BAG3
FS (%)
41
39
37
35
33
31
29
27
25
TAC (2)
Sham (2)
TAC + BAG3 (2)
Sham + BAG3 (1)
Presurgery
2W C
5 W
7 W
9 W (injection)
11 W
13 W
15 W
17 W

FIG. 4

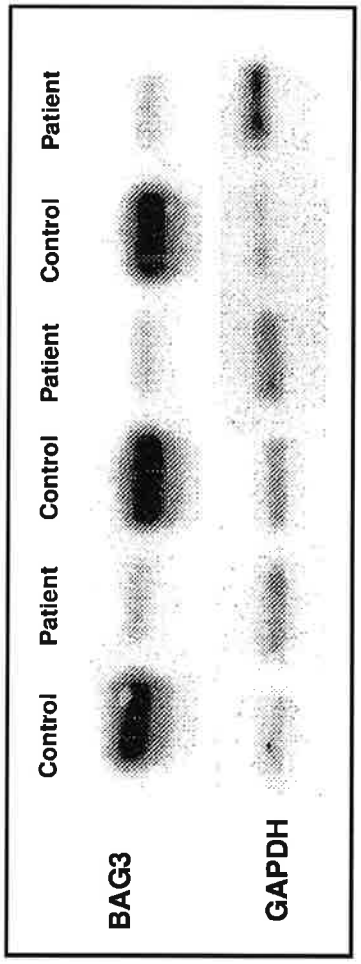

Control
Patient
Control
Patient
Control
Patient
BAG3
GAPDH

FIG. 5

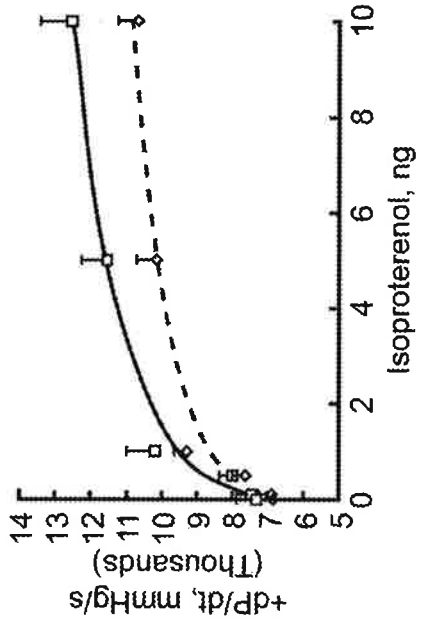

+dP/dt, mmHg/s
(Thousands)
14
13
12
11
10
9
8
7
6
5
0
2
4
6
8
10
Isoproterenol, ng

FIG. 6B

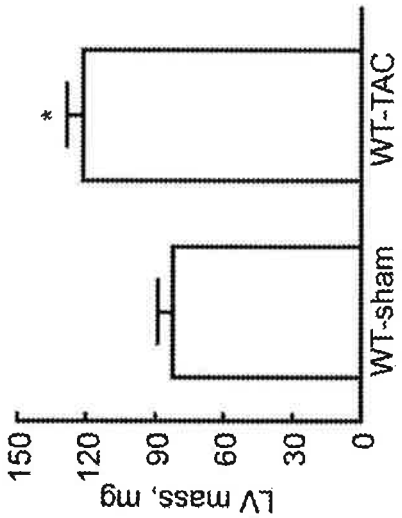

LV mass, mg
150
120
90
60
30
0
WT-sham
WT-TAC
*

FIG. 6A

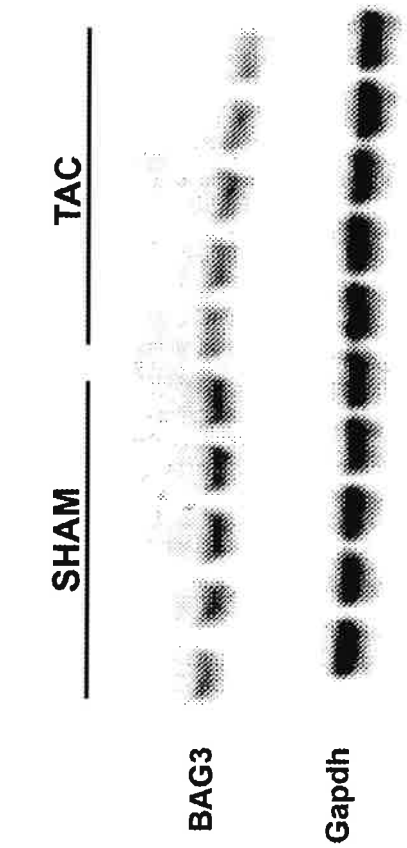

SHAM
TAC
BAG3
Gapdh

FIG. 6D

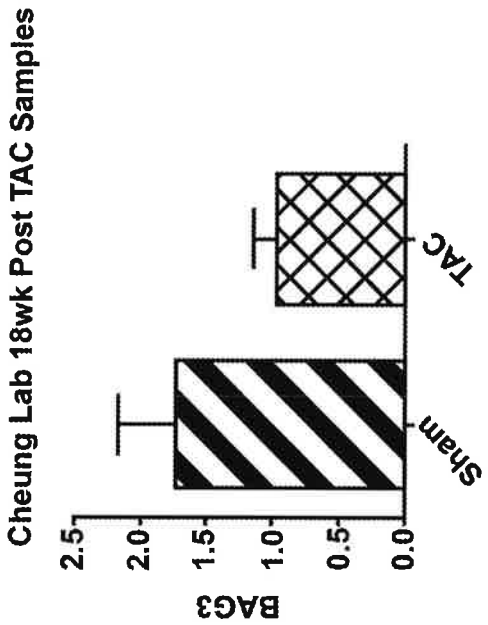
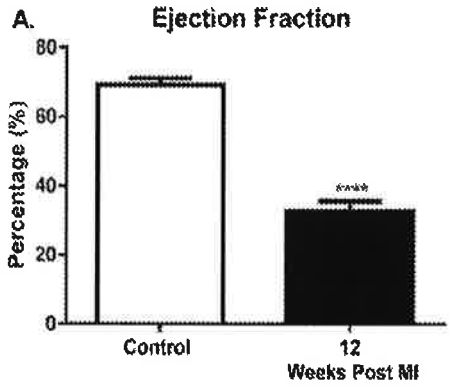

Cheung Lab 18wk Post TAC Samples
BAG3
2.5
2.0
1.5
1.0
0.5
0.0
Sham
TAC
Unpaired T-test P=0.0073

FIG. 6C

FIG. 7A


A.
Ejection Fraction
Percentage (%)
80
60
40
20
0
****
Control
12
Weeks Post MI

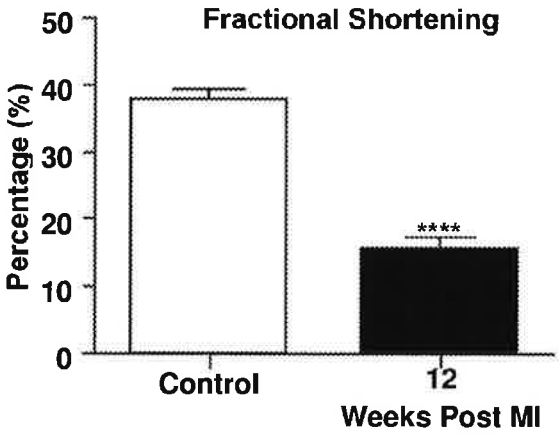

Fractional Shortening
Percentage (%)
50
40
30
20
10
0
****
Control
12
Weeks Post MI

FIG. 7B

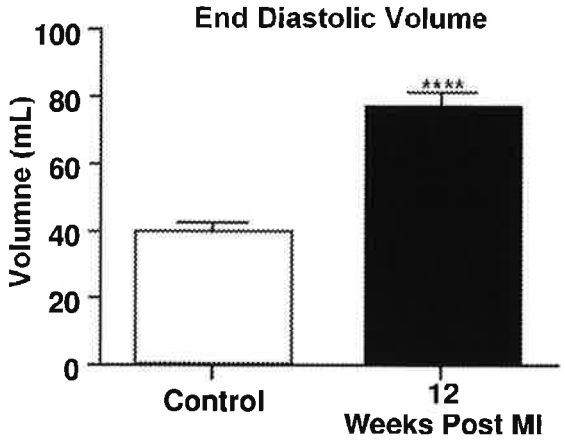

End Diastolic Volume
Volumne (mL)
100
80
60
40
20
0
****
Control
12
Weeks Post MI

FIG. 7C

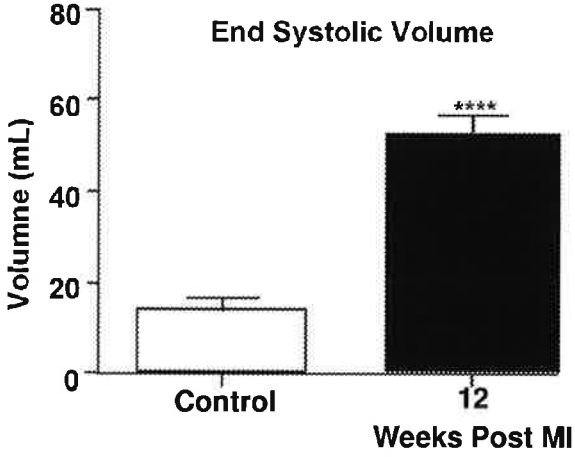

End Systolic Volume
Volumne (mL)
80
60
40
20
0
****
Control
12
Weeks Post MI

FIG. 7D

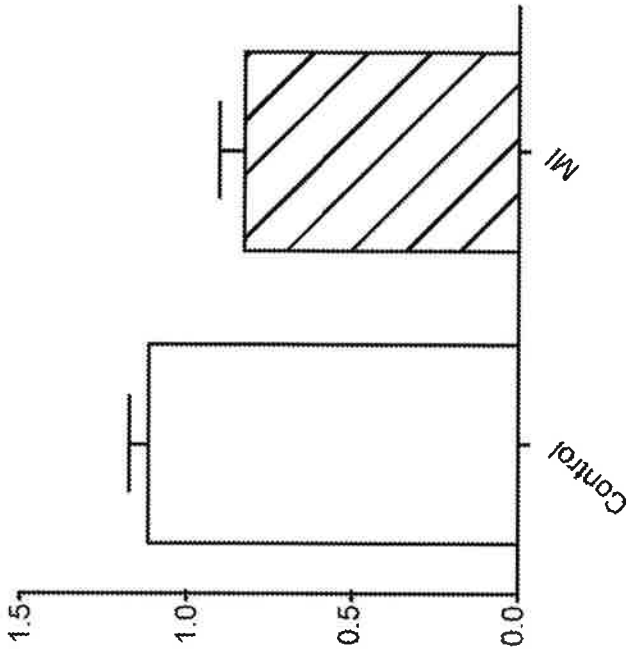

MI
Control
1.5
1.0
0.5
0.0

FIG. 7F

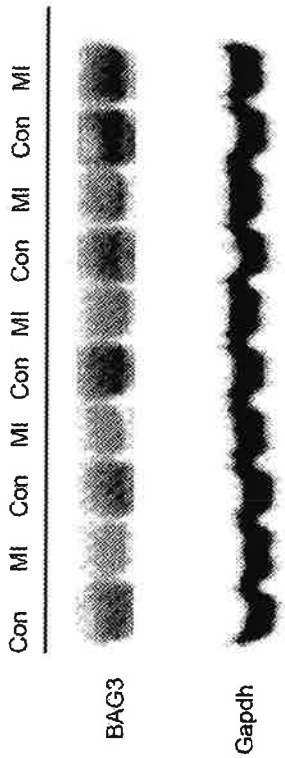

Con MI Con MI Con MI Con MI Con MI
BAG3
Gapdh

FIG. 7E

FIG. 8

Exemplary Human BAG3 Polypeptide Sequence
(Genbank NP_004272.2; Public GI:14043024

```
  1 msaathspmm qvasgngdrd plppgweiki dpqtgwpffv dhnsrtttwn dprvpsegpk
 61 etpssangps regsrlppar eghpvypqlr pgyipipvlh egaenrqvhp fhvypqpgmq
121 rfrteaaaaa pqrsqsplrg mpettqpdkq cgqvaaaaaa qppashgper sqspaasdcs
181 sssssaslps sgrsslgshq lprgyisipv iheqnvtrpa aqpsfhqaqk thypaqqgey
241 qthqpvyhki qgddweprpl raaspfrssv qgassregsp arsstplhsp spirvhtvvd
301 rpqqpmthre tapvsqpenk peskpgpvgp elppghipiq virkevdskp vsqkppppse
361 kvevkvppap vpcpppspgp savpssphsv ateeraapst apaeatppkp geaeappkhp
421 gvlkveaile kvqgleqavd nfegkktdkk ylmieeyltk ellaldsvdp egradvrqar
481 rdgvrkvqti leklaqkaid vpgqvqvyel qpsnleadqp lqaimemgav aadkqkknag
541 naedphtetq qpeataaats npssmtdtpg npaap (SEQ ID NO.1)
```

BAG3 AS A TARGET FOR THERAPY OF HEART FAILURE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application is a continuation application of U.S. application Ser. No. 15/115,807, filed Aug. 1, 2016, which is the National Phase of International Application No. PCT/US2015/013926, filed Jan. 30, 2015, which designated the U.S. and that International Application was published under PCT Article 21(2) in English, which claims the benefit of priority to U.S. Provisional Application No. 61/934,483, filed Jan. 31, 2014. The entire contents of the foregoing applications are incorporated herein by reference, including all text, tables and drawings.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with U.S. government support under grant number P01HL091799 awarded by the National Institutes of Health. The U.S. government may have certain rights in the invention.

SEQUENCE LISTING

[0003] This application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Sep. 10, 2020, is named "Sept20Renovacor-ST25.txt" and is 41 KB in size.

FIELD OF THE INVENTION

[0004] Embodiments of the invention are directed to compositions for the treatment of cardiac diseases or disorders, such as heart failure, cardiovascular diseases or disorders, or skeletal muscle diseases associated with Bcl-2 associated anthanogene-3 (BAG3) expression, and methods of treatment. Assays for the identification of novel therapeutic agents are also provided.

BACKGROUND

[0005] Heart failure (HF), secondary to systolic dysfunction and cardiac dilatation affects over 5 million individuals in the U.S. and is an important cause of both morbidity and mortality. Approximately 30% of these patients have non-ischemic disease or idiopathic dilated cardiomyopathy (IDC). Although in the majority of patients with IDC the causative factors have remained undefined, emerging evidence suggests that up to 35% of individuals with IDC have an affected first degree relative (Jefferies J L T J. *Lancet.* 2010; 375:752-762) and IDC can be associated with genetic abnormalities in 20-35% of individuals—leading to the use of the nomenclature familial dilated cardiomyopathy (FDC) (Judge D P et al, *Journal of Cardiovascular Translational Research.* 2008; 1:144-154; Hershberger R E et al. *Circulation. Cardiovascular Genetics.* 2010; 3:155-161). Indeed, mutations in more than 30 genes have been identified as causative factors (Hershberger R E, et al. *Circulation. Heart Failure.* 2009; 2:253-261) and the most common pattern of inheritance is autosomal dominant with reduced penetrance and variable expressivity (Morales A, Hershberger R E. *Current Cardiology Reports.* 2013; 15:375).

[0006] Mutations causing FDC are found in genes encoding a wide spectrum of proteins[6]; however, a large number of the mutations that cause FDC occur in genes that encode sarcomere proteins or the complex network of proteins in the Z-disc (Chang A N, Potter J D. *Heart Failure Reviews.* 2005; 10:225-235; Selcen D. Myofibrillar myopathies. *Neuromuscular disorders: NMD.* 2011; 21:161-171).

SUMMARY

[0007] Embodiments of the invention are directed to compositions for modulating the expression of Bcl-2 associated anthanogene-3 (BAG3) molecules, methods for identifying agents for treatment of cardiac diseases or disorders. In particular, these agents comprise expression vectors encoding Bcl-2 associated anthanogene-3 (BAG3) molecules, Bcl-2 associated anthanogene-3 (BAG3) nucleic acid sequences, Bcl-2 associated anthanogene-3 (BAG3) peptides or any other agent which modulates BAG3 expression. Such agents are identified by methods embodied herein. Conditions that are treated include, for example, heart failure, cardiomyopathy and the like. In some embodiments, the target tissues are cardiac tissues, such as for example, heart muscle.

[0008] Briefly, the results obtained herein have identified a rare and novel variant in a family with familial dilated cardiomyopathy. General embodiments of the invention are directed to treatment of patients identified as having variants of BAG3 molecules.

[0009] Patients with idiopathic dilated cardiomyopathy who did not have a mutation in the BAG3 gene were found to have half the normal level of BAG3, the same decrease that was found in the heart of the patient with the familial disease and the BAG3 mutation. Other general embodiments of the invention to treatment of patients with agents which modulate expression of BAG3 molecules, preferably resulting in overexpression of normal BAG3 molecules.

[0010] The results also showed that mice with heart failure due to aortic banding (a commonly used model for heart failure studies) had substantially less BAG3 than normal controls—and a reduction in BAG3 that mirrored that seen in humans.

[0011] Results also showed that when an AAV vector (AAV9) was administered in vivo, to over-express BAG3 in the heart, robust over-expression was observed. In other general embodiments, an agent comprises a cardiotropic vector expressing a BAG3 molecule. In some embodiments, the vector is an AAV9 vector.

[0012] It was also found that when the BAG3 protein was over-expressed in the hearts of mice with heart failure secondary to aortic banding (and low levels of BAG3) by using the AAV9 vector, normal left ventricular performance was reconstituted. These results provide evidence that BAG3 levels are decreased in the failing mouse heart and the AAV9 vector over-expressed BAG3 in the desired target in the heart resulting in the change in function comparable to a normal function.

[0013] Other aspects are described infra.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent

application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0015] FIG. **1** is a schematic representation showing the BAG3-Associated Dilated Cardiomyopathy Pedigree. Males are represented by squares. Circles indicate females. Open symbols represent unaffected individuals and black symbols represent affected individuals. The presence or absence of the 10-nucleotide deletion in BAG3 is indicated by either a (+) or a (−) respectively. An arrow denotes the proband. An asterisk is used to denote individuals whose DNA was used for whole exome sequencing. A diagonal line is used to denote individuals who are deceased.

[0016] FIG. **2**A is a schematic representation showing the sequencing alignment for BAG3 10-nucleotide deletion (SEQ ID NO:9-49). FIG. **2**B is a schematic representation showing the representative Sanger sequencing of the deletion in the BAG3 gene in an affected individual (SEQ ID NO:50-51).

[0017] FIGS. **3**A-**3**B show the low level expression of BAG3 in patients with heart failure. A representative Western blot of BAG3 and GAPDH levels in non-failing (NF) and failing (F) human heart is shown. The graph shows the quantification of BAG3 protein levels in non-failing and failing human heart. Values are normalized to the level of GAPDH in order to account for variations in protein loading. Horizontal lines represent mean and standard error of the mean. Statistical analysis was performed using unpaired t-test with Welch's correction for unequal variance.

[0018] FIG. **4** shows the measurement of ejection fraction in wild type sham operated mice, wild type mice that have been injected with the AAV9-BAG3 construct, mice that have undergone aortic banding (and developed heart failure) and mice that have been banded and in heart failure but were injected with AAV9-BAG3. As can be seen, the BAG3 injection normalized LV function temporally related to the expression of the BAG3 protein (approximately 5 to 6 weeks after injection).

[0019] FIG. **5** is a Western Blot showing BAG3 levels in patients. Three lanes of "control" and three lanes of "patient" are shown. This is patient IV-1—who is an affected. The "control" lanes are from a non-failing human heart—i.e. Normal human heart—that was obtained at the time of tissue harvest but could not be used for transplant because of size or tissue type incompatibility with available recipients. All of the lanes labeled "patient" were from the same patient—IV-1. These were obtained from pieces of his heart that were explanted at the time he underwent a heart transplant. The results show that the decrease in BAG3 levels are comparable to the decrease that was seen in the patients with non-familial heart failure.

[0020] FIGS. **6**A-**6**D show BAG3 levels in failing murine hearts. FIG. **6**A is a graph depicting heart weight to body weight ratios; FIG. **6**B is a graph depicting contractility; FIG. **6**C is a graph depicting BAG3 protein levels; FIG. **6**D is an immunoblotting analysis of BAG3 protein levels.

[0021] FIGS. **7**A-**7**F show hemodynamic indices and BAG3 levels in porcine hearts following balloon occlusion. FIG. **7**A is a graph depicting ejection fraction; FIG. **7**B is a graph depicting fractional shortening; FIG. **7**C is a graph depicting end diastolic volume; FIG. **7**D is a graph depicting end systolic volume; FIG. **7**E is a graph depicting BAG3 protein levels; FIG. **7**F is an immunoblotting analysis of BAG3 protein levels.

[0022] FIG. **8** shows the NCBI reference amino acid sequence for BAG3 (SEQ ID NO:1).

DETAILED DESCRIPTION

[0023] The present invention is based, in part, on the inventors' discovery of a novel BAG3 mutation in a family with adult-onset familial dilated cardiomyopathy (FDC). More specifically, the inventors have found that a novel 10 nucleotide deletion segregated in all affected individuals. Moreover, the inventors also found that levels of BAG3 protein were significantly reduced in hearts from unrelated patients with end-stage heart failure compared to non-failing controls. Further, the inventors have shown that, in a murine model of heart failure, administration of an AAV vector expressing BAG3, restored normal ventricular function. Accordingly, the invention features compositions that increase the expression of BAG3, methods of making such compositions, and methods of using such compositions to treat a subject, i.e., a patient suffering from dilated cardiomyopathy. Also featured are methods and compositions for diagnosis of heart failure, for example, idiopathic dilated cardiomyopathy (IDC).

[0024] Bcl-2 associated anthanogene-3 (BAG3), also known as BCL2-Associated Athanogene 3; MFM6; Bcl-2-Binding Protein Bis; CAIR-1; Docking Protein CAIR-1; BAG Family Molecular Chaperone Regulator 3; BAG-3; BCL2-Binding Athanogene 3; or BIS, is a cytoprotective polypeptide that competes with Hip-1 for binding to HSP 70. BAG3 function is illustrated in FIG. **8** and the mechanism for BAG3 involvement in cardiomyocyte function is illustrated in FIG. **9**. The NCBI reference amino acid sequence for BAG3 can be found at Genbank under accession number NP_004272.2; Public GI:14043024. We refer to the amino acid sequence of Genbank accession number NP_004272.2; Public GI: 14043024 as SEQ ID NO: 1 as shown in FIG. **10**. The NCBI reference nucleic acid sequence for BAG3 can be found at Genbank under accession number NM_004281.3 GI:62530382. We refer to the nucleic acid sequence of Genbank accession number NM_004281.3 GI:62530382 as (SEQ ID NO: 2). Other BAG3 amino acid sequences include, for example, without limitation, 095817.3 GI:12643665 (SEQ ID NO: 3); EAW49383.1 GI:119569768 (SEQ ID NO: 4); EAW49382.1 GI:119569767 (SEQ ID NO: 5); and CAE55998.1 GI:38502170 (SEQ ID NO: 6). The BAG3 polypeptide of the invention can be a variant of a polypeptide described herein, provided it retains functionality.

[0025] Vectors containing nucleic acids encoding a BAG3 polypeptide are provided herein.

[0026] A novel BAG3 mutation was identified in a family with adult-onset FDC. BAG3 protein levels were significantly decreased in unrelated patients with non-familial IDC providing evidence that altered levels of BAG3 protein participate in the progression of HE.

[0027] Embodiments are directed to compositions which modulate expression of Bcl-2 associated anthanogene-3 (BAG3) in vivo or in vitro. Modulation of BAG3 in patients in need of such therapy, include, patients with cardiac diseases or disorders, for example heart failure, or muscular-skeletal diseases or disorders. Embodiments are also directed to identification of novel compounds or agents which modulate BAG3 expression using assays which measure BAG3 expression.

[0028] Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. The present invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention.

[0029] Embodiments of the invention may be practiced without the theoretical aspects presented. Moreover, the theoretical aspects are presented with the understanding that Applicants do not seek to be bound by the theory presented.

[0030] All genes, gene names, and gene products disclosed herein are intended to correspond to homologs from any species for which the compositions and methods disclosed herein are applicable. Thus, the terms include, but are not limited to genes and gene products from humans and mice. It is understood that when a gene or gene product from a particular species is disclosed, this disclosure is intended to be exemplary only, and is not to be interpreted as a limitation unless the context in which it appears clearly indicates. Thus, for example, for the genes disclosed herein, which in some embodiments relate to mammalian nucleic acid and amino acid sequences are intended to encompass homologous and/or orthologous genes and gene products from other animals including, but not limited to other mammals, fish, amphibians, reptiles, and birds. In preferred embodiments, the genes or nucleic acid sequences are human.

Definitions

[0031] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[0032] As used herein, the terms "comprising," "comprise" or "comprised," and variations thereof, in reference to defined or described elements of an item, composition, apparatus, method, process, system, etc. are meant to be inclusive or open ended, permitting additional elements, thereby indicating that the defined or described item, composition, apparatus, method, process, system, etc. includes those specified elements—or, as appropriate, equivalents thereof—and that other elements can be included and still fall within the scope/definition of the defined item, composition, apparatus, method, process, system, etc.

[0033] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0034] "Optional" or "optionally" means that the subsequently described circumstance may or may not occur, such that the description includes instances where the circumstance occurs and instances where it does not.

[0035] The term "expression vector" as used herein refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules, siRNA, ribozymes, and the like. Expression vectors can contain a variety of control sequences, which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operatively linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well.

[0036] A "recombinant viral vector" refers to a viral vector comprising one or more heterologous gene products or sequences. Since many viral vectors exhibit size-constraints associated with packaging, the heterologous gene products or sequences are typically introduced by replacing one or more portions of the viral genome. Such viruses may become replication-defective, requiring the deleted function (s) to be provided in trans during viral replication and encapsidation (by using, e.g., a helper virus or a packaging cell line carrying gene products necessary for replication and/or encapsidation). Modified viral vectors in which a polynucleotide to be delivered is carried on the outside of the viral particle have also been described (see, e.g., Curiel, D T, et al., *PNAS* 88: 8850-8854, 1991).

[0037] By "encoding" or "encoded", "encodes", with respect to a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid, or may lack such intervening non-translated sequences (e.g., as in cDNA). The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the "universal" genetic code.

[0038] As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

[0039] A "constitutive promoter" is a promoter which drives expression of a gene to which it is operably linked, in a constant manner in a cell. By way of example, promoters

which drive expression of cellular housekeeping genes are considered to be constitutive promoters.

[0040] An "inducible" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only when an inducer which corresponds to the promoter is present in the cell.

[0041] A "tissue-specific" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

[0042] As used herein "BAG3", "BAG3 molecules", "BCL2-associated athanogene 3 (BAG3) genes", "BCL2-associated athanogene 3 (BAG3) molecules" are inclusive of all family members, mutants, cDNA sequences, alleles, fragments, species, coding and noncoding sequences, sense and antisense polynucleotide strands, etc. Similarly "BAG3", "BAG3 molecules", "BCL2-associated athanogene 3 (BAG3) molecules" also refer to BAG3 polypeptides or fragment thereof, proteins, variants, derivatives etc. The term "molecule", thus encompasses both the nucleic acid sequences and amino acid sequences of BAG3.

[0043] An "isolated nucleic acid or cDNA" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs, and refers to nucleic acid sequences in which one or more introns have been removed. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA, for instance, DNA which is part of a hybrid gene encoding additional polypeptide sequences.

[0044] A "polynucleotide" means a single strand or parallel and anti-parallel strands of a nucleic acid. Thus, a polynucleotide may be either a single-stranded or a double-stranded nucleic acid.

[0045] The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to a wild type gene. This definition may also include, for example, "allelic," "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. Of particular utility in the invention are variants of wild type gene products. Variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes that give rise to variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

[0046] Unless otherwise indicated, the terms "peptide", "polypeptide" or "protein" are used interchangeably herein, although typically they refer to peptide sequences of varying sizes.

[0047] "Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as "encoding" the protein or other product of that gene or cDNA.

[0048] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

[0049] A "non-natural amino acid" refers to an amino acid that is not one of the 20 common amino acids or pyrolysine or selenocysteine. Other terms that may be used synonymously with the term "non-natural amino acid" is "non-naturally encoded amino acid," "unnatural amino acid," "non-naturally-occurring amino acid," and variously hyphenated and non-hyphenated versions thereof. The term "non-natural amino acid" includes, but is not limited to, amino acids which occur naturally by modification of a naturally encoded amino acid (including but not limited to, the 20 common amino acids or pyrrolysine and selenocysteine) but are not themselves incorporated, without user manipulation, into a growing polypeptide chain by the translation complex. Examples of naturally-occurring amino acids that are not naturally-encoded include, but are not limited to, N-acetylglucosaminyl-L-serine, N-acetylglucosaminyl-L-threonine, and O-phosphotyrosine. Additionally, the term "non-natural amino acid" includes, but is not limited to, amino acids which do not occur naturally and may be obtained synthetically or may be obtained by modification of non-natural amino acids.

[0050] As used herein, the term "misexpression" refers to a non-wild type pattern of gene expression. It includes: expression at non-wild type levels, i.e., over- or underexpression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a

predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

[0051] By the term "modulate," it is meant that any of the mentioned activities of the compounds embodied herein, are, e.g., increased, enhanced, increased, agonized (acts as an agonist), promoted, decreased, reduced, suppressed blocked, or antagonized (acts as an antagonist). Modulation can increase activity more than 1-fold, 2-fold, 3-fold, 5-fold, 10-fold, 100-fold, etc., over baseline values. Modulation can also decrease its activity below baseline values.

[0052] As used herein, the term "agent" is meant to encompass any molecule, chemical entity, composition, drug, therapeutic agent, chemotherapeutic agent, or biological agent capable of preventing, ameliorating, or treating a disease or other medical condition. The term includes small molecule compounds, antisense reagents, siRNA reagents, antibodies, enzymes, peptides organic or inorganic molecules, natural or synthetic compounds and the like. An agent can be assayed in accordance with the methods of the invention at any stage during clinical trials, during pre-trial testing, or following FDA-approval.

[0053] As defined herein, a "therapeutically effective" amount of a compound or agent (i.e., an effective dosage) means an amount sufficient to produce a therapeutically (e.g., clinically) desirable result. The compositions can be administered one from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the compounds of the invention can include a single treatment or a series of treatments.

[0054] The terms "determining", "measuring", "evaluating", "detecting", "assessing" and "assaying" are used interchangeably herein to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assessing may be relative or absolute. "Assessing the presence of includes determining the amount of something present, as well as determining whether it is present or absent.

[0055] The term "assay" used herein, whether in the singular or plural shall not be misconstrued or limited as being directed to only one assay with specific steps but shall also include, without limitation any further steps, materials, various iterations, alternatives etc., that can also be used. Thus, if the term "assay" is used in the singular, it is merely for illustrative purposes.

[0056] A "label" or a "detectable label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radio labeled molecules fluorophores, luminescent compounds, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be made detectable, e.g., by incorporating a label into the peptide or used to detect antibodies specifically reactive with the peptide.

[0057] The term "high-throughput screening" or "HTS" refers to a method drawing on different technologies and disciplines, for example, optics, chemistry, biology or image analysis to permit rapid, highly parallel biological research and drug discovery. HTS methods are known in the art and they are generally performed in multiwell plates with automated liquid handling and detection equipment; however it is envisioned that the methods of the invention may be practiced on a microarray or in a microfluidic system.

[0058] The term "library" or "drug library" as used herein refers to a plurality of chemical molecules (test compound), a plurality of nucleic acids, a plurality of peptides, or a plurality of proteins, organic or inorganic compounds, synthetic molecules, natural molecules, or combinations thereof.

[0059] As used herein, the term "target" or "target molecule" refers to any type of molecule, or structure to be detected, manipulated or characterized. The molecule can be an intracellular molecule, such as for example, nucleic acid sequences, peptides, structures (e.g. intracellular membranes, ribosomes, etc.), surface molecules (e.g. receptors), extracellular molecules (e.g. cytokines, enzymes, viral particles, organisms, biological samples and the like.

[0060] As used herein, "biological samples" include solid and body fluid samples. The biological samples used in the present invention can include cells, protein or membrane extracts of cells, blood or biological fluids such as ascites fluid or brain fluid (e.g., cerebrospinal fluid). Examples of solid biological samples include, but are not limited to, samples taken from tissues of the central nervous system, bone, breast, kidney, cervix, endometrium, head/neck, gallbladder, parotid gland, prostate, pituitary gland, muscle, esophagus, stomach, small intestine, colon, liver, spleen, pancreas, thyroid, heart, lung, bladder, adipose, lymph node, uterus, ovary, adrenal gland, testes, tonsils, thymus and skin, or samples taken from tumors. Examples of "body fluid samples" include, but are not limited to blood, serum, semen, prostate fluid, seminal fluid, urine, feces, saliva, sputum, mucus, bone marrow, lymph, and tears.

[0061] As used herein, "cardiac disease" refers to any type of heart disease including heart failure, heart muscle disease, cardiomyopathy, hypertrophic cardiomyopathy, dilated cardiomyopathy, atherosclerosis, coronary artery disease, ischemic heart disease, myocarditis, viral infection, wounds, hypertensive heart disease, valvular disease, congenital heart disease, myocardial infarction, congestive heart failure, arrhythmias, diseases resulting in remodeling of the heart, etc. Diseases of the heart can be due to any reason, such as for example, damage to cardiac tissue such as a loss of contractility (e.g., as might be demonstrated by a decreased ejection fraction).

[0062] Cardiac damage or disorder characterized by insufficient cardiac function includes any impairment or absence of a normal cardiac function or presence of an abnormal cardiac function. Abnormal cardiac function can be the result of disease, injury, and/or aging. As used herein, abnormal cardiac function includes morphological and/or functional abnormality of a cardiomyocyte, a population of cardiomyocytes, or the heart itself. Non-limiting examples of morphological and functional abnormalities include

physical deterioration and/or death of cardiomyocytes, abnormal growth patterns of cardiomyocytes, abnormalities in the physical connection between cardiomyocytes, under- or over-production of a substance or substances by cardiomyocytes, failure of cardiomyocytes to produce a substance or substances which they normally produce, and transmission of electrical impulses in abnormal patterns or at abnormal times. Abnormalities at a more gross level include dyskinesis, reduced ejection fraction, changes as observed by echocardiography (e.g., dilatation), changes in EKG, changes in exercise tolerance, reduced capillary perfusion, and changes as observed by angiography. Abnormal cardiac function is seen with many disorders including, for example, ischemic heart disease, e.g., angina pectoris, myocardial infarction, chronic ischemic heart disease, hypertensive heart disease, pulmonary heart disease (cor pulmonale), valvular heart disease, e.g., rheumatic fever, mitral valve prolapse, calcification of mitral annulus, carcinoid heart disease, infective endocarditis, congenital heart disease, myocardial disease, e.g., myocarditis, dilated cardiomyopathy, hypertensive cardiomyopathy, cardiac disorders which result in congestive heart failure, and tumors of the heart, e.g., primary sarcomas and secondary tumors. Heart damage also includes wounds, such as for example, knife wound; biological (e.g. viral; autoimmune diseases) or chemical (e.g. chemotherapy, drugs); surgery; transplantation and the like.

[0063] As used herein the phrase "diagnostic" means identifying the presence or nature of a pathologic condition. Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of "true positives"). Diseased individuals not detected by the assay are "false negatives." Subjects who are not diseased and who test negative in the assay are termed "true negatives." The "specificity" of a diagnostic assay is 1 minus the false positive rate, where the "false positive" rate is defined as the proportion of those without the disease who test positive. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

[0064] As used herein the phrase "diagnosing" refers to classifying a disease or a symptom, determining a severity of the disease, monitoring disease progression, forecasting an outcome of a disease and/or prospects of recovery. The term "detecting" may also optionally encompass any of the above. Diagnosis of a disease according to the present invention can be effected by determining a level of a polynucleotide or a polypeptide of the present invention in a biological sample obtained from the subject, wherein the level determined can be correlated with predisposition to, or presence or absence of the disease. It should be noted that a "biological sample obtained from the subject" may also optionally comprise a sample that has not been physically removed from the subject, as described in greater detail below.

[0065] "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology or symptoms of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. "Treatment" may also be specified as palliative care. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. Accordingly, "treating" or "treatment" of a state, disorder or condition includes: (1) preventing or delaying the appearance of clinical symptoms of the state, disorder or condition developing in a human or other mammal that may be afflicted with or predisposed to the state, disorder or condition but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition; (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof (in case of maintenance treatment) or at least one clinical or subclinical symptom thereof; or (3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or subclinical symptoms. The benefit to an individual to be treated is either statistically significant or at least perceptible to the patient or to the physician.

[0066] The terms "patient" or "individual" or "subject" are used interchangeably herein, and refers to a mammalian subject to be treated, with human patients being preferred. In some cases, the methods of the invention find use in experimental animals, in veterinary application, and in the development of animal models for disease, including, but not limited to, rodents including mice, rats, and hamsters; and primates.

[0067] As used herein, the term "kit" refers to any delivery system for delivering materials. In the context of reaction assays, such delivery systems include systems that allow for the storage, transport, or delivery of reaction reagents (e.g., oligonucleotides, enzymes, etc. in the appropriate containers) and/or supporting materials (e.g., buffers, written instructions for performing the assay etc.) from one location to another. For example, kits include one or more enclosures (e.g., boxes) containing the relevant reaction reagents and/or supporting materials. As used herein, the term "fragmented kit" refers to a delivery systems comprising two or more separate containers that each contain a subportion of the total kit components. The containers may be delivered to the intended recipient together or separately. For example, a first container may contain an enzyme for use in an assay, while a second container contains oligonucleotides. The term "fragmented kit" is intended to encompass kits containing Analyte specific reagents (ASR's) regulated under section 520(e) of the Federal Food, Drug, and Cosmetic Act, but are not limited thereto. Indeed, any delivery system comprising two or more separate containers that each contains a subportion of the total kit components are included in the term "fragmented kit." In contrast, a "combined kit" refers to a delivery system containing all of the components of a reaction assay in a single container (e.g., in a single box housing each of the desired components). The term "kit" includes both fragmented and combined kits.

Compositions

[0068] The most common cause of dilated cardiomyopathy and heart failure (HF) is ischemic heart disease, however, in a third of all patients the cause remains undefined and patients are diagnosed as having idiopathic dilated cardiomyopathy (IDC). The studies conducted herein, employed whole-exome sequencing to identify the causative variant in a large family with autosomal dominant transmission of dilated cardiomyopathy. Sequencing and subsequent informatics revealed a novel 10-nucleotide deletion in the BCL2-associated athanogene 3 (BAG3) gene ((Ch10:del 121436332_12143641: del. 1266_1275 [NM 004281]) that segregated with all affected individuals. The deletion pre-

dicted a shift in the reading frame with the resultant deletion of 135 amino acids from the C-terminal end of the protein. Consistent with genetic variants in genes encoding other sarcomeric proteins there was a considerable amount of genetic heterogeneity in the affected family members. Interestingly, it was also found that the levels of BAG3 protein were significantly reduced in the hearts from unrelated patients with end-stage HF undergoing cardiac transplantation when compared with non-failing controls. Diminished levels of BAG3 protein may be associated with both familial and non-familial forms of dilated cardiomyopathy. Accordingly, modulation of expression of BAG3 or amounts of BAG3 in a patient would be of great benefit.

[0069] In embodiments, a therapeutic agent for treatment of diseases associated with BAG3 and associated molecules and pathways thereof, modulates the expression or amounts of BAG3 in a cell.

[0070] In some embodiments, compositions comprise nucleic acid sequences of BCL2-associated athanogene 3 (BAG3), including without limitation, cDNA, sense and/or antisense sequences of BAG3.

[0071] In some embodiments, a composition comprises an expression vector having an isolated nucleic acid or cDNA sequence or synthetic nucleic acid sequence, encoding BCL2-associated athanogene 3 (BAG3) molecules. The term "nucleic acid sequence" will be used for the sake of brevity and will include, without limitation, isolated nucleic acid or cDNA sequences, synthesized or synthetic nucleic acid sequences, chimeric nucleic acid sequences, homologs, orthologs, variants, mutants or combinations thereof.

[0072] In some embodiments, a nucleic acid sequence of BAG3 comprises at least about a 50% sequence identity to wild type BAG3 or cDNA sequences thereof. In other embodiments, the BAG3 nucleic acid sequence comprises at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to wild type BAG3 or cDNA sequences thereof.

[0073] In some embodiments, a nucleic acid sequence of BAG3 further comprises one or more mutations, substitutions, deletions, variants or combinations thereof.

[0074] In some embodiments, the homology, sequence identity or complementarity, between a BAG3 nucleic acid sequence comprising one or more mutations, substitutions, deletions, variants or combinations thereof and the native or wild type or cDNA sequences of BAG3 is from about 50% to about 60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

[0075] In one embodiment, an expression vector encodes a BCL2-associated athanogene 3 (BAG3) gene or cDNA sequences thereof, or modified sequences thereof. In one embodiment, the expression vector encodes a nucleic acid sequence comprising at least about 50% sequence identity to wild type BCL2-associated athanogene 3 (BAG3) or cDNA sequences thereof. In other embodiments, the nucleic acid sequence comprises at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to wild type BCL2-associated athanogene 3 (BAG3) or cDNA sequences thereof.

[0076] A wide variety of host/expression vector combinations may be employed in expressing the BAG3 DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col E1, pCR1, pBR322, pMal-C2, pET, pGEX (Smith et al., Gene 67:31-40, 1988), pMB9 and their derivatives, plasmids such as RP4; phage DNAs, e.g., the numerous derivatives of phage 1, e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof, vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

[0077] A number of vectors are known to be capable of mediating transfer of gene products to mammalian cells, as is known in the art and described herein. A "vector" (sometimes referred to as gene delivery or gene transfer "vehicle") refers to a macromolecule or complex of molecules comprising a polynucleotide to be delivered to a host cell, either in vitro or in vivo. The polynucleotide to be delivered may comprise a coding sequence of interest in gene therapy. Vectors include, for example, viral vectors (such as adenoviruses ("Ad"), adeno-associated viruses (AAV), and vesicular stomatitis virus (VSV) and retroviruses), liposomes and other lipid-containing complexes, and other macromolecular complexes capable of mediating delivery of a polynucleotide to a host cell. Vectors can also comprise other components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. As described and illustrated in more detail below, such other components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector nucleic acid by the cell; components that influence localization of the polynucleotide within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of the polynucleotide. Such components also might include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities. Other vectors include those described by Chen et al; *BioTechniques*, 34: 167-171 (2003). A large variety of such vectors are known in the art and are generally available.

[0078] Suitable nucleic acid delivery systems include viral vector, typically sequence from at least one of an adenovirus, adenovirus-associated virus (AAV), helper-dependent adenovirus, retrovirus, or hemagglutinating virus of Japan-liposome (HVJ) complex. Preferably, the viral vector comprises a strong eukaryotic promoter operably linked to the polynucleotide e.g., a cytomegalovirus (CMV) promoter.

[0079] Additionally preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include Moloney murine leukemia viruses and HIV-based viruses. One HIV-based viral vector comprises at least two vectors wherein the gag and pol genes are from an HIV genome and the env gene is from another virus. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector [Geller, A. I. et al., *J. Neurochem,* 64: 487 (1995); Lim, F., et al., in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); Geller, A. I. et al., *Proc Natl. Acad. Sci.*: U.S.A.:90 7603 (1993); Geller, A. I., et al., *Proc Natl. Acad. Sci* USA: 87:1149 (1990)], Adenovirus Vectors [LeGal LaSalle et al., *Science,* 259:988 (1993); Davidson, et al., *Nat. Genet.* 3: 219 (1993); Yang, et al., *J. Virol.* 69: 2004 (1995)] and Adeno-associated Virus Vectors [Kaplitt, M. G., et al., *Nat. Genet.* 8:148 (1994)].

[0080] Pox viral vectors introduce the gene into the cells cytoplasm. Avipox virus vectors result in only a short term expression of the nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus (HSV) vectors may be an indication for some invention embodiments. The adenovirus vector results in a shorter term expression (e.g., less than about a month) than adeno-associated vims (AAV), in some embodiments, may exhibit much longer expression. In some embodiments, the expression vector is an AAV9 vector. The particular vector chosen will depend upon the target cell and the condition being treated. The selection of appropriate promoters can readily be accomplished. Preferably, one would use a high expression promoter. An example of a suitable promoter is the 763-base-pair cytomegalovirus (CMV) promoter. The Rous sarcoma vims (RSV) (Davis, et al., *Hum Gene Ther* 4:151 (1993)) and MMT promoters may also be used. Certain proteins can expressed using their native promoter. Other elements that can enhance expression can also be included such as an enhancer or a system that results in high levels of expression such as a tat gene and tar element. This cassette can then be inserted into a vector, e.g., a plasmid vector such as, pUC19, pUC118, pBR322, or other known plasmid vectors, that includes, for example, an *E. coli* origin of replication. See, Sambrook, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory press, (1989). The plasmid vector may also include a selectable marker such as the β-lactamase gene for ampicillin resistance, provided that the marker polypeptide does not adversely affect the metabolism of the organism being treated. The cassette can also be bound to a nucleic acid binding moiety in a synthetic delivery system, such as the system disclosed in WO 95/22618.

[0081] If desired, the polynucleotides of the invention may also be used with a microdelivery vehicle such as cationic liposomes and adenoviral vectors. For a review of the procedures for liposome preparation, targeting and delivery of contents, see Mannino and Gould-Fogerite, *BioTechniques,* 6:682 (1988). See also, Feigner and Holm, *Bethesda Res. Lab. Focus,* 11(2):21 (1989) and Maurer, R. A., *Bethesda Res. Lab. Focus,* 11(2):25 (1989).

[0082] Replication-defective recombinant adenoviral vectors, can be produced in accordance with known techniques. See, Quantin, et al., *Proc. Natl. Acad. Sci.* USA, 89:2581-2584 (1992); Stratford-Perricadet, et al., J. Clin. Invest., 90:626-630 (1992); and Rosenfeld, et al., Cell, 68:143-155 (1992).

[0083] Another delivery method is to use single stranded DNA producing vectors which can produce the BAG3 intracellularly, for example, cardiac tissues. See for example, Chen et al, *BioTechniques,* 34: 167-171 (2003), which is incorporated herein, by reference, in its entirety.

[0084] Expression of BAG3 may be controlled by any promoter/enhancer element known in the art, but these regulatory elements must be functional in the host selected for expression. In some embodiments, the promoter is a tissue specific promoter. Of particular interest are muscle specific promoters, and more particularly, cardiac specific promoters. These include the myosin light chain-2 promoter (Franz et al. (1994) *Cardioscience,* Vol. 5(4):235-43; Kelly et al. (1995) *J. Cell Biol.*, Vol. 129(2):383-396), the alpha actin promoter (Moss et al. (1996) *Biol. Chem.,* Vol. 271 (49):31688-31694), the troponin 1 promoter (Bhaysar et al. (1996) *Genomics*, Vol. 35(1): 11-23); the Na^+/Ca^{2+} exchanger promoter (Barnes et al. (1997) J. Biol. Chem., Vol. 272(17): 11510-11517), the dystrophin promoter (Kimura et al. (1997) *Dev. Growth Differ.*, Vol. 39(3):257-265), the alpha7 integrin promoter (Ziober and Kramer (1996) *J. Bio. Chem.*, Vol. 271(37):22915-22), the brain natriuretic peptide promoter (LaPointe et al. (1996) *Hypertension*, Vol. 27(3 Pt 2):715-22) and the alpha B-crystallin/small heat shock protein promoter (Gopal-Srivastava (1995) *J. Mol. Cell. Biol.*, Vol. 15(12):7081-7090), alpha myosin heavy chain promoter (Yamauchi-Takihara et al. (1989) *Proc. Natl. Acad. Sci.* USA, Vol. 86(10):3504-3508) and the ANF promoter (LaPointe et al. (1988) *J. Biol. Chem.*, Vol. 263(19):9075-9078).

[0085] Other promoters which may be used to control BAG3 gene expression include, but are not limited to, cytomegalovirus (CMV) promoter (U.S. Pat. Nos. 5,385,839 and 5,168,062), the SV40 early promoter region (Benoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., *Cell* 22:787-797, 1980), the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci.* U.S.A. 78:1441-1445, 1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39-42, 1982); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., *Proc. Natl. Acad. Sci.* U.S.A. 75:3727-3731, 1978), or the tac promoter (DeBoer, et al., *Proc. Natl. Acad. Sci.* U.S.A. 80:21-25, 1983); see also "Useful proteins from recombinant bacteria" in Scientific American, 242:74-94, 1980; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., *Cell* 38:639-646, 1984; Omitz et al., Cold Spring Harbor Symp. *Quant. Biol.* 50:399-409, 1986; MacDonald, *Hepatology* 7:425-515, 1987); insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature* 315:115-122, 1985), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., Cell 38:647-658, 1984; Adames et al., *Nature* 318:533-538, 1985; Alexander et al., *Mol. Cell. Biol.* 7:1436-1444, 1987), mouse mammary

tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., *Cell* 45:485-495, 1986), albumin gene control region which is active in liver (Pinkert et al., *Genes and Devel.* 1:268-276, 1987), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., *Mol. Cell. Biol.* 5:1639-1648, 1985; Hammer et al., *Science* 235:53-58, 1987), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., *Genes and Devel.* 1:161-171, 1987), beta-globin gene control region which is active in myeloid cells (Mogram et al., *Nature* 315:338-340, 1985; Kollias et al., *Cell* 46:89-94, 1986), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., *Cell* 48:703-712, 1987), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature* 314:283-286, 1985), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., *Science* 234:1372-1378, 1986).

[0086] Yeast expression systems can also be used according to the invention to express BAG3. For example, the non-fusion pYES2 vector (XbaI, SphI, ShoI, NotI, GstXI, EcoRI, BstXI, BamHI, SacI, KpnI, and HindIII cloning sites; Invitrogen) or the fusion pYESHisA, B, C (XbaI, SphI, ShoI, NotI, BstXI, EcoRI, BamHI, SacI, KpnI, and HindIII cloning sites, N-terminal peptide purified with ProBond resin and cleaved with enterokinase; Invitrogen), to mention just two, can be employed according to the invention. A yeast two-hybrid expression system can be prepared in accordance with the invention.

[0087] One preferred delivery system is a recombinant viral vector that incorporates one or more of the polynucleotides therein, preferably about one polynucleotide. Preferably, the viral o vector used in the invention methods has a pfu (plague forming units) of from about 10^8 to about 5×10^{10} pfu. In embodiments in which the polynucleotide is to be administered with a non-viral vector, use of between from about 0.1 nanograms to about 4000 micrograms will often be useful e.g., about 1 nanogram to about 100 micrograms.

[0088] In some embodiments, the vector is an adenovirus-associated viral vector (AAV), for example, AAV9. The term "AAV vector" means a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7 and AAV-8. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes, but retain functional flanking ITR sequences. Despite the high degree of homology, the different serotypes have tropisms for different tissues. The receptor for AAV 1 is unknown; however, AAV 1 is known to transduce skeletal and cardiac muscle more efficiently than AAV2. Since most of the studies have been done with pseudotyped vectors in which the vector DNA flanked with AAV2 ITR is packaged into capsids of alternate serotypes, it is clear that the biological differences are related to the capsid rather than to the genomes. Recent evidence indicates that DNA expression cassettes packaged in AAV 1 capsids are at least 1 log 10 more efficient at transducing cardiomyocytes than those packaged in AAV2 capsids. In one embodiment, the viral delivery system is an adeno-associated viral delivery system. The adeno-associated virus can be of serotype I (AAV 1), serotype 2 (AAV2), serotype 3 (AAV3), serotype 4 (AAV4), serotype 5 (AAV5), serotype 6 (AAV6), serotype 7 (AAV7), serotype 8 (AAV8), or serotype 9 (AAV9).

[0089] Some skilled in the art have circumvented some of the limitations of adenovirus-based vectors by using adenovirus "hybrid" viruses, which incorporate desirable features from adenovirus as well as from other types of viruses as a means of generating unique vectors with highly specialized properties. For example, viral vector chimeras were generated between adenovirus and adeno-associated virus (AAV). These aspects of the invention do not deviate from the scope of the invention described herein.

[0090] Nucleic acids encoding the BAG3 proteins of the invention may be delivered to cardiac muscle by methods known in the art (see e.g., US Patent Appln. Publication No. US 2009/0209631). For example, cardiac cells of a large mammal may be transfected by a method that includes dilating a blood vessel of the coronary circulation by administering a vasodilating substance to said mammal prior to, and/or concurrent with, administering the nucleic acids. In some embodiments, the method includes administering the nucleic acids into a blood vessel of the coronary circulation in vivo, wherein nucleic acids are infused into the blood vessel over a period of at least about three minutes, wherein the coronary circulation is not isolated or substantially isolated from the systemic circulation of the mammal, and wherein the nucleic acids transfect cardiac cells of the mammal.

[0091] In some embodiments, the subject can be a human, an experimental animal, e.g., a rat or a mouse, a domestic animal, e.g., a dog, cow, sheep, pig or horse, or a non-human primate, e.g., a monkey. The subject may be suffering from a cardiac disorder, such as heart failure, ischemia, myocardial infarction, congestive heart failure, arrhythmia, transplant rejection and the like. In a preferred embodiment, the subject is suffering from heart failure. In another particular embodiment, the subject is suffering from arrhythmia. In one embodiment, the subject is a human. For example, the subject is between ages 18 and 65. In another embodiment, the subject is a non-human animal.

[0092] In one embodiment, the subject has or is at risk for heart failure, e.g. a non-ischemic cardiomyopathy, mitral valve regurgitation, ischemic cardiomyopathy, or aortic stenosis or regurgitation.

[0093] In some embodiments, transfection of cardiac cells with nucleic acid molecules encoding a BAG3 protein or BAG3 protein fused to an effector domain increases lateral ventricle fractional shortening. In some embodiments, the mammal is human and the disease is congestive heart failure. In some embodiments, the transfection of the cardiac cells increases lateral ventricle fractional shortening when measured about 4 months after said infusion by at least 25% as compared to lateral ventricle fractional shortening before infusion of the polynucleotide. In some embodiments, the transfection of the cardiac cells results in an improvement in a measure of cardiac function selected from the group consisting of expression of BAG3 protein, fractional shortening, ejection fraction, cardiac output, time constant of ventricular relaxation, and regurgitant volume.

[0094] A treatment can be evaluated by assessing the effect of the treatment on a parameter related to contractility. For example, SR Ca2+ATPase activity or intracellular Ca^{2+} concentration can be measured. Furthermore, force generation by hearts or heart tissue can be measured using methods described in Strauss et al., *Am. J. Physiol.*, 262:1437-45, 1992, the contents of which are incorporated herein by reference.

[0095] Modified Nucleic Acid Sequences:

[0096] It is not intended that the present invention be limited by the nature of the nucleic acid employed, as long as they modulate the expression or quantities of BAG3 in a cell, or patient to whom, the nucleic acid composition is to be administered as a therapeutic agent. The nucleic acid may be DNA or RNA and may exist in a double-stranded, single-stranded or partially double-stranded form.

[0097] Nucleic acids useful in the present invention include, by way of example and not limitation, oligonucleotides and polynucleotides such as antisense DNAs and/or RNAs; ribozymes; DNA for gene therapy; viral fragments including viral DNA and/or RNA; DNA and/or RNA chimeras; mRNA; plasmids; cosmids; genomic DNA; cDNA; gene fragments; various structural forms of DNA including single-stranded DNA, double-stranded DNA, supercoiled DNA and/or triple-helical DNA; Z-DNA; and the like. The nucleic acids may be prepared by any conventional means typically used to prepare nucleic acids in large quantity. For example, DNAs and RNAs may be chemically synthesized using commercially available reagents and synthesizers by methods that are well-known in the art (see, e.g., Gait, 1985, OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH (IRE Press, Oxford, England)). RNAs may be produce in high yield via in vitro transcription using plasmids such as pGEM® T vector or SP65 (Promega Corporation, Madison, Wis.).

[0098] Accordingly, certain preferred nucleic acid sequences of this invention are chimeric nucleic acid sequences. "Chimeric nucleic acid sequences" or "chimeras," in the context of this invention, contain two or more chemically distinct regions, each made up of at least one nucleotide. These sequences typically contain at least one region of modified nucleotides that confers one or more beneficial properties (such as, for example, increased nuclease resistance, increased uptake into cells, increased binding affinity for the target).

[0099] Chimeric nucleic acid sequences of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures comprise, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

[0100] Specific examples of some modified nucleic acid sequences envisioned for this invention include those comprising modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Examples of oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, include without limitation: CH_2—NH—O—CEH_2, CH, —$N(CH_3)$—O—CH_2 [known as a methylene(methylimino) or MMI backbone], CH_2—O—$N(CH_3)$—CH_2, CH_2—$N(CH_3)$—$N(CH_3)$—CH_2 and O—$N(CH_3)$—CH—CH_2 backbones, wherein the native phosphodiester backbone is represented as O—P—O—CH,). The amide backbones disclosed by De Mesmaeker et al. (1995) *Acc. Chem. Res.* 28:366-374 are also one example. In other embodiments, a nucleic acid sequence comprises morpholino backbone structures (Summerton and Weller, U.S. Pat. No. 5,034,506). In other embodiments, such as the peptide nucleic acid (PNA) backbone, the phosphodiester backbone of the nucleic acid sequence is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone (Nielsen et al. (1991) Science 254, 1497). Nucleic acid sequences may also comprise one or more substituted sugar moieties. Examples include: OH, SH, SCH_3, F, OCN, OCH_3 OCH_3, OCH3 $O(CH_2)_nCH_3$, $O(CH_2)_nNH_2$ or $O(CH_2)_n$ CH_3 where n is from 1 to about 10; C_1 to C_{10} lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF_3; OCF_3; O—, S—, or N-alkyl; O—, S—, or N-alkenyl; $SOCH_3$; SO_2 CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; poly alky lamino; substituted silyl; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. Other modifications include, for example: 2'-methoxyethoxy [2, —O—CH_2 CH_2 OCH_3, also known as 2'—O—(2-methoxyethyl)] (Martin et al., (1995) *Hely. Chin. Acta,* 78, 486), 2'-methoxy (2'—O—CH_3), 2,-propoxy (2'—OCH_2 CH_2CH_3) and 2'-fluoro (2'-F). Similar modifications may also be made at any positions on the oligonucleotide, the 2' or the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. The nucleic acid sequences may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

[0101] Preferred modified oligonucleotide backbones comprise, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3' alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising S'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[0102] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl intemucleoside linkages, mixed heteroatom and alkyl or cycloalkyl intemucleoside linkages, or one or more short chain heteroatomic or heterocyclic intemucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[0103] The nucleic acid sequences may also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleotides include adenine (A), guanine (G), thymine (T), cytosine (C) and

uracil (U). Modified nucleotides include nucleotides found only infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxy methyl cytosine (HMC), glycosyl HMC and gentobiosyl HMC, as well as synthetic nucleotides, e.g., 2-aminoadenine, 2-(methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalklyamino)adenine or other hetero-substituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N6 (6-aminohexyl)adenine and 2,6-diaminopurine. (Komberg, A., DNA Replication, W.H. Freeman & Co., San Francisco, 1980, pp 75-77; Gebeyehu, G., (1987) et al. *Nucl. Acids Res.* 15:4513). A "universal" base known in the art, e.g., inosine, may be included.

[0104] Another modification involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, a cholesteryl moiety, cholic acid, a thioether, e.g., hexyl-5-tritylthiol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid. Nucleic acid sequences comprising lipophilic moieties, and methods for preparing such oligonucleotides are known in the art, for example, U.S. Pat. Nos. 5,138,045, 5,218,105 and 5,459,255.

[0105] It is not necessary for all positions in a given nucleic acid sequence to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single nucleic acid sequence or even at within a single nucleoside within an such sequences. The present invention also includes oligonucleotides which are chimeric oligonucleotides as hereinbefore defined.

[0106] In another embodiment, the BAGS nucleic acid molecule of the present invention is conjugated with another moiety including but not limited to abasic nucleotides, polyether, polyamine, polyamides, peptides, carbohydrates, lipid, or polyhydrocarbon compounds. Those skilled in the art will recognize that these molecules can be linked to one or more of any nucleotides comprising the nucleic acid molecule at several positions on the sugar, base or phosphate group.

[0107] In another embodiment, the BAG3 nucleic acid sequences comprise one or more nucleotides substituted with locked nucleic acids (LNA). The LNA modified nucleic acid sequences may have a size similar to the parent or native sequence or may be larger or preferably smaller. It is preferred that such LNA-modified oligonucleotides contain less than about 70%, more preferably less than about 60%, most preferably less than about 50% LNA monomers and that their sizes are between about 1 and 25 nucleotides.

[0108] Antisense BAG3-Oligonucleotides:

[0109] In another preferred embodiment, the expression of BAG3 in a cell or patient is modulated by one or more target nucleic acid sequences which modulate the expression of BAG3, for example, transcriptional regulator elements.

[0110] In a preferred embodiment, an oligonucleotide comprises at least five consecutive bases complementary to a nucleic acid sequence, wherein the oligonucleotide specifically hybridizes to and modulates expression of BAG3 in vivo or in vitro. In another preferred embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenosine, variants may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the oligonucleotide. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

[0111] In some embodiments, homology, sequence identity or complementarity, between the oligonucleotide and target is from about 50% to about 60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

[0112] In another preferred embodiment, an oligonucleotide comprises combinations of phosphorothioate internucleotide linkages and at least one internucleotide linkage selected from the group consisting of: alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and/or combinations thereof.

[0113] In another preferred embodiment, an oligonucleotide optionally comprises at least one modified nucleobase comprising, peptide nucleic acids, locked nucleic acid (LNA) molecules, analogues, derivatives and/or combinations thereof.

[0114] An oligonucleotide is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target nucleic acid sequences under conditions in which specific binding is desired. Such conditions include, i.e., physiological conditions in the case of in vivo assays or therapeutic treatment, and conditions in which assays are performed in the case of in vitro assays.

[0115] An oligonucleotide, whether DNA, RNA, chimeric, substituted etc, is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarily to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

[0116] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that

can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

[0117] In embodiments of the present invention oligomeric oligonucleotides, particularly oligonucleotides, bind to target nucleic acid molecules and modulate the expression of molecules encoded by a target gene. The functions of DNA to be interfered comprise, for example, replication and transcription. The functions of RNA to be interfered comprise all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The functions may be up-regulated or inhibited depending on the functions desired.

[0118] The oligonucleotides, include, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

[0119] Targeting an oligonucleotide to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. This target nucleic acid may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state.

[0120] The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, e.g., modulation of expression, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in the present invention, are defined as positions within a target nucleic acid.

[0121] In another preferred embodiment, the antisense oligonucleotides bind to coding and/or non-coding regions of a target polynucleotide and modulate the expression and/or function of the target molecule.

[0122] In another preferred embodiment, the antisense oligonucleotides bind to natural antisense polynucleotides and modulate the expression and/or function of the target molecule. An example of a "function" can be one which inhibits a negative regulator of transcription, thus allowing for an increased expression of a desired molecule, such as, for example, BAGS.

[0123] In another preferred embodiment, the antisense oligonucleotides bind to sense polynucleotides and modulate the expression and/or function of the target molecule.

[0124] In embodiments of the invention the oligonucleotides bind to an antisense strand of a particular target. The oligonucleotides are at least 5 nucleotides in length and can be synthesized so each oligonucleotide targets overlapping sequences such that oligonucleotides are synthesized to cover the entire length of the target polynucleotide. The targets also include coding as well as non coding regions.

[0125] According to the present invention, antisense compounds include antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleobase positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines, however, in preferred embodiments, the gene expression is up regulated. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

[0126] In another preferred embodiment, the desired oligonucleotides or antisense compounds, comprise at least one of: antisense RNA, antisense DNA, chimeric antisense oligonucleotides, antisense oligonucleotides comprising modified linkages, interference RNA (RNAi), short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof.

[0127] dsRNA can also activate gene expression, a mechanism that has been termed "small RNA-induced gene activation" or RNAa. dsRNAs targeting gene promoters induce potent transcriptional activation of associated genes. RNAa was demonstrated in human cells using synthetic dsRNAs, termed "small activating RNAs" (saRNAs).

[0128] Small double-stranded RNA (dsRNA) may also act as small activating RNAs (saRNA). Without wishing to be bound by theory, by targeting sequences in gene promoters, saRNAs would induce target gene expression in a phenomenon referred to as dsRNA-induced transcriptional activation (RNAa).

[0129] In some embodiments, the ribonucleic acid sequence is specific for regulatory segments of the genome that control the transcription of BAG3. Thus a candidate therapeutic agent can be a dsRNA that activates the expression of BAG3 in a cell and is administered to a patient in need of treatment.

[0130] Peptides:

[0131] In another embodiment, a BAG3 peptide is encoded by a nucleic acid comprising a BCL2-associated athanogene 3 (BAG3) wild type, chimeric or cDNA sequences thereof. The peptide can also be a synthetic peptide of BCL2-associated athanogene 3 (BAG3).

[0132] It is to be understood that the peptide sequences are not limited to the native or cDNA sequences thereof, of BCL2-associated athanogene 3 (BAG3) molecules. The skilled artisan will recognize that conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function. Conservative amino acid substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, lysine, arginine, phenylalanine, tyrosine.

[0133] Conservative substitutions may also be made based on types of amino acids: aliphatic (valine, isoleucine, leucine, and alanine); charged (aspartic acid, glutamic acid, lysine, arginine, and histidine); aromatic residues (phenylalanine, tyrosine and tryptophan); and sulfur-containing (methionine and cysteine). Polypeptide sequences having at least about 68% identity, at least about 70% identity, or at least about 71% identity to a BCL2-associated athanogene 3 (BAG3) nucleic acid sequence, or cDNA sequences thereof.

[0134] The determination of percent identity between two nucleotide or amino acid sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, *Proc. Natl. Acad. Sci. USA* 87:2264-2268), modified as in Karlin and Altschul (1993, *Proc. Natl. Acad. Sci.* USA 90:5873-5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, *J. Mol. Biol.* 215:403-410), and can be accessed, for example at the National Center for Biotechnology Information (NCBI) world wide web site having the universal resource locator http://blast(dot)ncbi(dot)nlm(dot)nih(dot)gov/blast.cgi/. BLAST nucleotide searches can be performed with the NBLAST program (designated "blastn" at the NCBI web site), using the following parameters: gap penalty=5; gap extension penalty=2; mismatch penalty=3; match reward=1; expectation value 10.0; and word size=11 to obtain nucleotide sequences homologous to a nucleic acid described herein. BLAST protein searches can be performed with the XBLAST program or the NCBI "blastp" program, using the following parameters: expectation value 10.0, BLOSUM62 scoring matrix to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997, *Nucleic Acids Res.* 25:3389-3402). Alternatively, PSI-Blast or PHI-Blast can be used to perform an iterated search which detects distant relationships between molecules and relationships between molecules which share a common pattern. When utilizing BLAST, Gapped BLAST, PSI-Blast, and PHI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. In calculating percent identity, exact matches are typically counted.

[0135] Embodiments of the invention also include polynucleotides encoding hybrid proteins comprising BCL2-associated athanogene 3 (BAG3) polypeptide operatively fused directly or indirectly via peptide linker, to a second polypeptide sequence. Linker sequences are well known in the art. In one embodiment, a hybrid protein comprises a BAG3 polypeptide or a BAG3 polypeptide operatively fused to a detectable moiety, such as, a reporter polypeptide, wherein the reporter polypeptide is fused to the N- or C-terminal of the BAG3 polypeptide, directly or indirectly. Exemplary reporter polypeptides include luciferase (LUC), green fluorescent protein (GFP), and GFP derivatives.

[0136] Hybrid proteins comprising a BAG3 polypeptide or fragment thereof may be linked to other types of polypeptides, in addition to a reporter polypeptide, or in lieu of a reporter polypeptide. These additional polypeptides may be any amino acid sequence useful for the purification, identification, and/or therapeutic or prophylactic application of the peptide. In addition, the additional polypeptide can be a signal peptide, or targeting peptide, etc.

[0137] In some cases, the other additions, substitutions or deletions may increase the stability (including but not limited to, resistance to proteolytic degradation) of the polypeptide or increase affinity of the polypeptide for its appropriate receptor, ligand and/or binding proteins. In some cases, the other additions, substitutions or deletions may increase the solubility of the polypeptide. In some embodiments sites are selected for substitution with a naturally encoded or non-natural amino acid in addition to another site for incorporation of a non-natural amino acid for the purpose of increasing the polypeptide solubility following expression in recombinant host cells. In some embodiments, the polypeptides comprise another addition, substitution, or deletion that modulates affinity for the associated ligand, binding proteins, and/or receptor, modulates (including but not limited to, increases or decreases) receptor dimerization, stabilizes receptor dimers, modulates circulating half-life, modulates release or bio-availability, facilitates purification, or improves or alters a particular route of administration. Similarly, the non-natural amino acid polypeptide can comprise chemical or enzyme cleavage sequences, protease cleavage sequences, reactive groups, antibody-binding domains (including but not limited to, FLAG or poly-His) or other affinity based sequences (including but not limited to, FLAG, poly-His, GST, etc.) or linked molecules (including but not limited to, biotin) that improve detection (including but not limited to, GFP), purification, transport through tissues or cell membranes, prodrug release or activation, size reduction, or other traits of the polypeptide.

[0138] The methods and compositions described herein include incorporation of one or more non-natural amino acids into a polypeptide. One or more non-natural amino acids may be incorporated at one or more particular positions which does not disrupt activity of the polypeptide. This can be achieved by making "conservative" substitutions, including but not limited to, substituting hydrophobic amino acids with non-natural or natural hydrophobic amino acids, bulky amino acids with non-natural or natural bulky amino acids, hydrophilic amino acids with non-natural or natural hydrophilic amino acids) and/or inserting the non-natural amino acid in a location that is not required for activity.

[0139] A variety of biochemical and structural approaches can be employed to select the desired sites for substitution with a non-natural amino acid within the polypeptide. Any position of the polypeptide chain is suitable for selection to incorporate a non-natural amino acid, and selection may be based on rational design or by random selection for any or no particular desired purpose. Selection of desired sites may be based on producing a non-natural amino acid polypeptide (which may be further modified or remain unmodified) having any desired property or activity, including but not limited to agonists, super-agonists, partial agonists, inverse agonists, antagonists, receptor binding modulators, receptor activity modulators, modulators of binding to binder partners, binding partner activity modulators, binding partner conformation modulators, dimer or multimer formation, no change to activity or property compared to the native molecule, or manipulating any physical or chemical property of the polypeptide such as solubility, aggregation, or stability. For example, locations in the polypeptide required for biological activity of a polypeptide can be identified using methods including, but not limited to, point mutation analysis, alanine scanning or homolog scanning methods. Residues other than those identified as critical to biological activity by methods including, but not limited to, alanine or homolog scanning mutagenesis may be good candidates for substitution with a non-natural amino acid depending on the desired activity sought for the polypeptide. Alternatively, the sites identified as critical to biological activity may also be good candidates for substitution with a non-natural amino acid, again depending on the desired activity sought for the polypeptide. Another alternative would be to make serial substitutions in each position on the polypeptide chain with a non-natural amino acid and observe the effect on the activities of the polypeptide. Any means, technique, or method for selecting a position for substitution with a non-natural amino acid into any polypeptide is suitable for use in the methods, techniques and compositions described herein.

Candidate Agents and Screening Assays

[0140] The compositions embodied herein, can also be applied in the areas of drug discovery and target validation. The present invention comprehends the use of the nucleic acid sequences and peptides embodied herein, in drug discovery efforts to elucidate relationships that exist between Bcl-2 associated anthanogene-3 (BAG3) polynucleotides and a disease state, phenotype, or condition. These methods include detecting or modulating Bcl-2 associated anthanogene-3 (BAG3) polynucleotides comprising contacting a sample, tissue, cell, or organism with a compound, measuring the nucleic acid or protein level of Bcl-2 associated anthanogene-3 (BAG3) polynucleotides and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further compound of the invention.

[0141] The screening assays of the invention suitably include and embody, animal models, cell-based systems and non-cell based systems. The nucleic acid sequences and peptides embodied herein, are used for identifying agents of therapeutic interest, e.g. by screening libraries of compounds or otherwise identifying compounds of interest by any of a variety of drug screening or analysis techniques, or synthesis of novel compounds. The gene, allele, fragment, or oligopeptide thereof employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The measurements are conducted as described in detail in the examples section which follows. In embodiments, screening candidate agents is performed to identify those which modulate the translation of BAG3.

[0142] The assays can be of an in vitro or in vivo format. In vitro formats of interest include cell-based formats, in which contact occurs e.g., by introducing the substrate in a medium, such as an aqueous medium, in which the cell is present. In yet other embodiments, the assay may be in vivo, in which a multicellular organism that includes the cell is employed. Contact of a targeting vector encoding the nucleic acid sequences embodied herein, with the target cell(s) may be accomplished using any convenient protocol. In those embodiments where the target cells are present as part of a multicellular organism, e.g., an animal, the vector is conveniently administered to (e.g., injected into, fed to, etc.) the multicellular organism, e.g., a whole animal, where administration may be systemic or localized, e.g., directly to specific tissue(s) and/or organ(s) of the multicellular organism.

[0143] Multicellular organisms of interest include, but are not limited to: insects, vertebrates, such as avian species, e.g., chickens; mammals, including rodents, e.g., mice, rates; ungulates, e.g., pigs, cows, horses; dogs, cats, primates, e.g., monkeys, apes, humans; and the like. As such, the target cells of interest include, but are not limited to: insects cells, vertebrate cells, particularly avian cells, e.g., chicken cells; mammalian cells, including murine, porcine, ungulate, ovine, equine, rat, dog, cat, monkey, and human cells; and the like.

[0144] The target cell comprising the BAG3 polynucleotides or BAG3 polypeptides is contacted with a test compound and the translation of BAG3 is evaluated or assessed by detecting the presence or absence of signal from a detectable moiety, for example, luciferase substrate, i.e., by screening the cell (either in vitro or in vivo) for the presence of a luciferase mediated luminescent signal. The detected signal is then employed to evaluate the translational and/or transcriptional activity of BAG3 in the presence of a test agent.

[0145] The luminescent signal may be detected using any convenient luminescent detection device. In certain embodiments, detectors of interest include, but are not limited to: photo-multiplier tubes (PMTs), avalanche photodiodes (APDs), charge-coupled devices (CCDs); complementary metal oxide semiconductors (CMOS detectors) and the like. The detector may be present in a signal detection device, e.g., luminometer, which is capable of detecting the signal once or a number of times over a predetermined period, as desired. Data may be collected in this way at frequent intervals, for example once every 10 ms, over the course of a given assay time period.

[0146] In certain embodiments, the subject methods are performed in a high throughput (HT) format. In the subject HT embodiments of the subject invention, a plurality of different cells are simultaneously assayed or tested. By simultaneously tested is meant that each of the cells in the plurality are tested at substantially the same time. In general, the number of cells that are tested simultaneously in the subject HT methods ranges from about 10 to 10,000, usually from about 100 to 10,000 and in certain embodiments from

about 1000 to 5000. A variety of high throughput screening assays for determining the activity of candidate agent are known in the art and are readily adapted to the present invention, including those described in e.g., Schultz (1998) *BioorgMed Chem Lett* 8:2409-2414; Fernandes (1998) *Curr Opin Chem Biol* 2:597-603; as well as those described in U.S. Pat. No. 6,127,133; the disclosures of which are herein incorporated by reference.

[0147] In some embodiments, a method of screening for agents which modulate translation and/or transcription of Bcl-2 associated anthanogene-3 (BAG3) comprises contacting a BAG3 molecule with an agent wherein the BAG3 molecule comprises an isolated nucleic acid or cDNA sequence of Bcl-2 associated anthanogene-3 (BAG3) operably linked to a detectable moiety, and at least one stop codon between the BAG3 and the detectable moiety; assessing the level of translation of the BAG3 in the absence of a candidate agent to obtain a reference level of translation and/or transcription, assessing the level of translation and/or transcription of BAG3 in the presence of the candidate agent to obtain a test level of translation and/or transcription, wherein the candidate agent is identified as an agent that increases translation and/or transcription if the test level of translation and/or transcription is greater than the reference level of translation and/or transcription.

[0148] In embodiments, the detectable moiety comprises: a luminescent moiety, a chemiluminescent moiety, a fluorescence moiety, a bioluminescent moiety, an enzyme, a natural or synthetic moiety.

[0149] Any method known in the art can be used to assess translation. In a preferred embodiment, translation is assessed using mammalian cells transfected with an expression vector comprising a nucleic acid of the invention. The transfection may be transient or the cells may stably transformed with the expression vector. A cell-based assay such as described in Butcher et al., 2007, *J Biol Chem.* 282:2853-28539 may be used. Alternatively, an in vitro translation assay may be used.

[0150] In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast or insect cell, by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical or biological means.

[0151] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, photoporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

[0152] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0153] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (i.e., an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

[0154] In the case where a non-viral delivery system is utilized, a preferred delivery vehicle is a liposome. The above-mentioned delivery systems and protocols therefore can be found in "Gene Targeting Protocols, 2ed.", Kmiec ed., Humana Press, Totowa, N.J., pp 1-35 (2002) and "Gene Transfer and Expression Protocols, Vol. 7, (Methods in Molecular Biology)," Murray ed., Humana Press, Totowa, N.J., pp 81-89 (1991).

[0155] Candidate Agents:

[0156] The methods can be practiced with any test compounds as candidate agents. Test compounds useful in practicing the inventive method may be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries, spatially-addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, nonpeptide oligomer, or small molecule libraries of compounds (Lam, 1997, *Anticancer Drug Des.* 12:145).

[0157] Examples of methods for the synthesis of molecular libraries may be found in the art, for example, in: DeWitt et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:6909-6913; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Zuckermann et al., 1994, *J. Med. Chem.* 37:2678-2685; Cho et al., 1992, *Science* 261:1303-1305; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2059-2061; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2061-2064; and Gallop et al., 1994, *J. Med. Chem.* 37:1233-1251.

[0158] Libraries of compounds may be presented in solution (e.g., Houghten, 1992, *Bio/Techniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria (U.S. Pat. No. 5,223,409), spores (U.S. Pat. Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:1865-1869), or phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, *Science* 249:404-406; Cwirla et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:6378-6382; and Felici, 1991, *J Mol. Biol.* 222:301-310).

[0159] Commercially available libraries that may be screened include, but are not limited to, the TimTec Natural Product Library (NPL), NPL-640, and TimTec NDL-3000 library. Libraries comprising compounds modeled on polyamines (i.e., polyamine analogs) may also be screened.

[0160] In certain embodiments, the candidate agent is a small molecule or large molecule ligand. By small molecule ligand is meant a ligand ranging in size from about 50 to about 10,000 daltons, usually from about 50 to about 5,000 daltons and more usually from about 100 to about 1000 daltons. By large molecule is meant a ligand ranging in size from about 10,000 daltons or greater in molecular weight.

[0161] The method may be practiced iteratively using different concentrations of a test candidate and/or different testing conditions, such as duration of reaction time. Test candidates that are identified by the method can be further tested by conventional methods in the art to verify speci-

ficity, dose dependency, efficacy in vivo, and the like. Test candidates may serve as lead compounds for developing additional test candidates.

[0162] As indicated above, the present invention finds use in monitoring translational and/or transcriptional activity of BAG3 in an assay wherein the test is conducted using cells. In these embodiments, the cells are cultured under specific user-defined conditions (e.g., in the presence or absence of a cytokine, nutrient and/or candidate therapeutic agent), and monitored for emitted light.

[0163] A prototype compound or agent may be believed to have therapeutic activity on the basis of any information available to the artisan. For example, a prototype agent may be believed to have therapeutic activity on the basis of information contained in the Physician's Desk Reference. In addition, by way of non-limiting example, a compound may be believed to have therapeutic activity on the basis of experience of a clinician, structure of the compound, structural activity relationship data, EC50, assay data, IC50 assay data, animal or clinical studies, or any other basis, or combination of such bases.

[0164] A therapeutically-active compound or agent is an agent that has therapeutic activity, including for example, the ability of the agent to induce a specified response when administered to a subject or tested in vitro. Therapeutic activity includes treatment of a disease or condition, including both prophylactic and ameliorative treatment. Treatment of a disease or condition can include improvement of a disease or condition by any amount, including prevention, amelioration, and elimination of the disease or condition. Therapeutic activity may be conducted against any disease or condition, including in a preferred embodiment against any disease or disorder associated with damage by reactive oxygen intermediates. In order to determine therapeutic activity any method by which therapeutic activity of a compound may be evaluated can be used. For example, both in vivo and in vitro methods can be used, including for example, clinical evaluation, EC_{50}, and IC_{50} assays, and dose response curves.

[0165] Candidate compounds for use with an assay of the present invention or identified by assays of the present invention as useful pharmacological agents can be pharmacological agents already known in the art or variations thereof or can be compounds previously unknown to have any pharmacological activity. The candidate compounds can be naturally occurring or designed in the laboratory. Candidate compounds can comprise a single diastereomer, more than one diastereomer, or a single enantiomer, or more than one enantiomer.

[0166] Candidate compounds can be isolated, from microorganisms, animals or plants, for example, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, candidate compounds of the present invention can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries. The other four approaches are applicable to polypeptide, non-peptide oligomers, or small molecule libraries of compounds and are preferred approaches in the present invention. See Lam, *Anticancer Drug Des.* 12: 145-167 (1997).

[0167] In an embodiment, the present invention provides a method of identifying a candidate compound as a suitable prodrug. A suitable prodrug includes any prodrug that may be identified by the methods of the present invention. Any method apparent to the artisan may be used to identify a candidate compound as a suitable prodrug.

[0168] In another aspect, the present invention provides methods of screening candidate compounds for suitability as therapeutic agents. Screening for suitability of therapeutic agents may include assessment of one, some or many criteria relating to the compound that may affect the ability of the compound as a therapeutic agent. Factors such as, for example, efficacy, safety, efficiency, retention, localization, tissue selectivity, degradation, or intracellular persistence may be considered. In an embodiment, a method of screening candidate compounds for suitability as therapeutic agents is provided, where the method comprises providing a candidate compound identified as a suitable prodrug, determining the therapeutic activity of the candidate compound, and determining the intracellular persistence of the candidate compound. Intracellular persistence can be measured by any technique apparent to the skilled artisan, such as for example by radioactive tracer, heavy isotope labeling, or LCMS.

[0169] In screening compounds for suitability as therapeutic agents, intracellular persistence of the candidate compound is evaluated. In a preferred embodiment, the agents are evaluated for their ability to modulate the translation of compositions embodied herein, over a period of time in response to a candidate therapeutic agent.

[0170] In another preferred embodiment, soluble and/or membrane-bound forms of compositions embodied herein, e.g. proteins, mutants or biologically active portions thereof, can be used in the assays for screening candidate agents. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, TRITON™ X-100, TRITON™ X-114, THESIT™, Isotridecypoly(ethylene glycol ether)n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

[0171] Cell-free assays can also be used and involve preparing a reaction mixture which includes BAG3 molecules (nucleic acids or peptides) comprising a bioluminescent moiety and the test compound under conditions and time periods to allow the measurement of the translational and/or transcriptional activity over time, and concentrations of test agents.

[0172] In other embodiments, a candidate agent is an antisense oligonucleotide. In embodiments, BAG3 expression (e.g., protein) in a sample (e.g., cells or tissues in vivo or in vitro) treated using an antisense oligonucleotide of the invention is evaluated by comparison with BAG3 expression in a control sample. For example, the translation of the BAG3 is monitored by the signal emitted by the detectable moiety and compared with that in a mock-treated or untreated sample. Alternatively, comparison with a sample treated with a control antisense oligonucleotide (e.g., one

having an altered or different sequence) can be made depending on the information desired. In another embodiment, a difference in the translational and/or transcriptional activity in a treated vs. an untreated sample can be compared with the difference in expression of a different nucleic acid (including any standard deemed appropriate by the researcher, e.g., a housekeeping gene) in a treated sample vs. an untreated sample.

[0173] Observed differences can be expressed as desired, e.g., in the form of a ratio or fraction, for use in a comparison with control. In some embodiments, the level of BAG3 protein, in a sample treated with an antisense oligonucleotide, is increased or decreased by about 1.25-fold to about 10-fold or more relative to an untreated sample or a sample treated with a control nucleic acid. Preferably, the level or amount of BAG3 is increased. In embodiments, the level of BAG3 protein is increased or decreased by at least about 1.25-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, or at least about 10-fold or more. In embodiments, it is preferable that the level or amount of BAG3 is increased.

[0174] Microarrays:

[0175] Identification of a nucleic acid sequence capable of binding to a target molecule can be achieved by immobilizing a library of nucleic acids onto the substrate surface so that each unique nucleic acid is located at a defined position to form an array. In general, the immobilized library of nucleic acids are exposed to a biomolecule or candidate agent under conditions which favored binding of the biomolecule to the nucleic acids. The nucleic acid array would then be analyzed by the methods embodied herein to determine which nucleic acid sequences bound to the biomolecule. Preferably the biomolecules would carry a pre-determined label for use in detection of the location of the bound nucleic acids.

[0176] An assay using an immobilized array of BAG3 nucleic acid sequences may be used for determining the sequence of an unknown nucleic acid; single nucleotide polymorphism (SNP) analysis; analysis of BAG3 gene expression patterns from a particular species, tissue, cell type, etc.; gene identification; etc.

[0177] In further embodiments, oligonucleotides or longer fragments derived from any of the BAG3 polynucleotide sequences, may be used as targets in a microarray. The microarray can be used to monitor the identity and/or expression level of large numbers of genes and gene transcripts simultaneously to identify genes with which target genes or its product interacts and/or to assess the efficacy of candidate therapeutic agents in regulating expression products of genes that mediate, for example, neurological disorders. This information may be used to determine gene function, and to develop and monitor the activities of therapeutic agents.

[0178] Microarrays may be prepared, used, and analyzed using methods known in the art (see, e.g., Brennan et al., 1995, U.S. Pat. No. 5,474,796; Schena et al., 1996, *Proc. Natl. Acad. Sci.* U.S.A. 93: 10614-10619; Baldeschweiler et al., 1995, PCT application WO95/251116; Shalon, et al., 1995, PCT application WO95/35505; Heller et al., 1997, *Proc. Natl. Acad. Sci. U.S.A.* 94: 2150-2155; and Heller et al., 1997, U.S. Pat. No. 5,605,662). In other embodiments, a microarray comprises BAG3 peptides, or other desired molecules which can be assayed to identify a candidate agent.

[0179] In another preferred embodiment a method for screening candidate agents for the treatment or prevention of a cardiac disease or disorder comprises contacting a sample with a candidate therapeutic agent and measuring the effects the agent has on a target. For example, the agent may regulate BAG3 expression and the agent can then be further studied for any possible therapeutic effects (increase or decrease parameter being monitored e.g. expression). An abnormal expression state may be caused by pathology such as heart failure, disease, cancer, genetic defects and/or a toxin.

[0180] Antibodies.

[0181] Useful diagnostic assays can include one or more antibodies that specifically bind BAG3. In some embodiments, the antibody specifically binds a mutant BAG3, for example, the BAG3 polypeptide disclosed herein having the 10 amino acid deletion as shown in FIG. **2**. We use the term antibody to broadly refer to immunoglobulin-based binding molecules, and the term encompasses conventional antibodies (e.g., the tetrameric antibodies of the G class (e.g., an IgG1)), fragments thereof that retain the ability to bind their intended target (e.g., an Fab' fragment), and single chain antibodies (scFvs). The antibody may be polyclonal or monoclonal and may be produced by human, mouse, rabbit, sheep or goat cells, or by hybridomas derived from these cells. The antibody can be humanized, chimeric, or bi-specific.

[0182] The antibodies can assume various configurations and encompass proteins consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Any one of a variety of antibody structures can be used, including the intact antibody, antibody multimers, or antibody fragments or other variants thereof that include functional, antigen-binding regions of the antibody. We may use the term "immunoglobulin" synonymously with "antibody." The antibodies may be monoclonal or polyclonal in origin. Regardless of the source of the antibody, suitable antibodies include intact antibodies, for example, IgG tetramers having two heavy (H) chains and two light (L) chains, single chain antibodies, chimeric antibodies, humanized antibodies, complementary determining region (CDR)-grafted antibodies as well as antibody fragments, e.g. Fab, Fab', F(ab')2, scFv, Fv, and recombinant antibodies derived from such fragments, e.g., camelbodies, microantibodies, diabodies and bispecific antibodies.

[0183] An intact antibody is one that comprises an antigen-binding variable region (V_H and V_L) as well as a light chain constant domain (C_L) and heavy chain constant domains, C_{H1}, C_{H2} and C_{H3}. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variants thereof. As is well known in the art, the V_H and V_L regions are further subdivided into regions of hypervariability, termed "complementarity determining regions" (CDRs), interspersed with the more conserved framework regions (FRs).

[0184] An anti-BAGS antibody can be from any class of immunoglobulin, for example, IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof (e.g., IgG_1, IgG_2, IgG_3, and IgG_4)), and the light chains of the immunoglobulin may be of types kappa or lambda. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA_1 and IgA_2), gamma (IgG_1, IgG_2, IgG_3, IgG_4), delta, epsilon, and mu constant region genes, as well as the many immunoglobulin variable region genes.

[0185] The term "antigen-binding portion" of an immunoglobulin or antibody refers generally to a portion of an immunoglobulin that specifically binds to a target, in this case, an epitope comprising amino acid residues on a BAG3 polypeptide. An antigen-binding portion of an immunoglobulin is therefore a molecule in which one or more immunoglobulin chains are not full length, but which specifically binds to a cellular target. Examples of antigen-binding portions or fragments include: (i) an Fab fragment, a monovalent fragment consisting of the VLC, VHC, CL and CHI domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fv fragment consisting of the VLC and VHC domains of a single arm of an antibody, and (v) an isolated CDR having sufficient framework to specifically bind, e.g., an antigen binding portion of a variable region. An antigen-binding portion of a light chain variable region and an antigen binding portion of a heavy chain variable region, e.g., the two domains of the Fv fragment, VLC and VHC, can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VLC and VHC regions pair to form monovalent molecules (known as single chain Fv (scFv). Such scFvs can be a target agent of the present invention and are encompassed by the term "antigen-binding portion" of an antibody.

[0186] An "Fv" fragment is the minimum antibody fragment that contains a complete antigen-recognition and binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, con-covalent association. It is in this configuration that three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. While six hypervariable regions confer antigen-binding specificity, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. To improve stability, the VH-VL domains may be connected by a flexible peptide linker such as $(GlY_4Ser)_3$ (SEQ ID NO:7) to form a single chain Fv or scFV antibody fragment or may be engineered to form a disulfide bond by introducing two cysteine residues in the framework regions to yield a disulfide stabilized Fv (dsFv). Fragments of antibodies are suitable for use in the methods provided so long as they retain the desired specificity of the full-length antibody and/or sufficient specificity to specifically bind to a BAG3 polypeptide.

[0187] The compositions of the present invention include antibodies that (1) exhibit a threshold level of binding activity; and/or (2) do not significantly cross-react with known related polypeptide molecules. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, *Ann. NY Acad, Sci.* 51:660-672 (1949)).

[0188] In some embodiments, the anti-BAG3 antibodies can bind to their target epitopes or mimetic decoys at least 1.5-fold, 2-fold, 5-fold 10-fold, 100-fold, 10^3-fold, 10^4-fold, 10^5-fold, 10^6-fold or greater for the target anti-BAG3 than to other proteins predicted to have some homology to BAG3.

[0189] In some embodiments the anti-BAG3 antibodies bind with high affinity of 10^{-4} M or less, 10^{-7} M or less, 10^{-9} M or less or with subnanomolar affinity (0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 nM or even less). In some embodiments the binding affinity of the anti-BAG3 antibodies for their respective targets is at least 1×10^6 Ka. In some embodiments the binding affinity of the anti-BAG3 antibodies for BAG3 is at least 5×10^6 Ka, at least 1×10^7 Ka, at least 2×10^7 Ka, at least 1×10^8 Ka, or greater. Antibodies may also be described or specified in terms of their binding affinity to BAG3. In some embodiments binding affinities include those with a Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-3} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M, or less.

[0190] In some embodiments, the antibodies do not bind to known related polypeptide molecules; for example, they bind BAG3, but not known related polypeptides. In some embodiments, the antibodies specifically bind to a mutant BAG3 polypeptide, for example a BAG3 polypeptide having the ten base pair deletion as shown in FIG. **2**, but not to a wild type BAG3 polypeptide. Antibodies may be screened against known related polypeptides to isolate an antibody population that specifically binds BAG3.

[0191] The diagnostic assays of the invention can include concurrent immunoelectrophoresis, radioimmunoassay (RIA), radioimmunoprecipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. The anti-BAG3 antibodies can include a tag, which may also be referred to as a reporter or marker (e.g., a detectable marker). A detectable marker can be any molecule that is covalently linked to the anti-BAG3 antibody or a biologically active fragment thereof that allows for qualitative and/or quantitative assessment of the expression or activity of the tagged peptide. The activity can include a biological activity, a physico-chemical activity, or a combination thereof. Both the form and position of the detectable marker can vary, as long as the labeled antibody retains biological activity. Many different markers can be used, and the choice of a particular marker will depend upon the desired application. Labeled anti-BAG3 antibodies can be used, for example, for assessing the levels of BAG3 or a mutant BAG3 in a biological sample, e.g., urine, saliva, cerebrospinal fluid, blood or a biopsy sample or for evaluation the clinical response to a cardiovascular therapeutic, for example, the BAG3 constructs described above.

[0192] Exemplary detectable labels include a radiopaque or contrast agents such as barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, and thallous chloride. Alternatively or in addition, the detectable label can be a fluorescent label, for example,

fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine; a chemiluminescent compound selected from the group consisting of luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester; a liposome or dextran; or a bioluminescent compound such as luciferin, luciferase and aequorin.

[0193] Suitable markers include, for example, enzymes, photo-affinity ligands, radioisotopes, and fluorescent or chemiluminescent compounds. Methods of introducing detectable markers into peptides are well known in the art. Markers can be added during synthesis or post-synthetically. Recombinant anti-BAG3 antibodies or biologically active variants thereof can also be labeled by the addition of labeled precursors (e.g., radiolabeled amino acids) to the culture medium in which the transformed cells are grown. In some embodiments, analogues or variants of peptides can be used in order to facilitate incorporation of detectable markers. For example, any N-terminal phenylalanine residue can be replaced with a closely related aromatic amino acid, such as tyrosine, that can be easily labeled with 125I. In some embodiments, additional functional groups that support effective labeling can be added to the fragments of an anti-BAG3 antibody or biologically active variant thereof. For example, a 3-tributyltinbenzoyl group can be added to the N-terminus of the native structure; subsequent displacement of the tributyltin group with 125I will generate a radiolabeled iodobenzoyl group.

[0194] Any art-known method can be used for detecting such labels, for example, positron-emission tomography (PET), SPECT imaging, magnetic resonance imaging, X-ray; or is detectable by ultrasound.

[0195] In other preferred embodiments, a method of treating a patient having a cardiac disease or disorder, wherein the patient has decreased BAG3 levels as compared to a baseline level, comprising administering a pharmaceutical composition comprising a therapeutically effective amount of at least one BAG3 inducing agent wherein the agent increases expression of the BAG3 molecule.

[0196] In other embodiments, a method of preventing or treating a subject at risk of or suffering from a cardiac disease or disorder comprising: administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide. In preferred embodiments the cardiac disease and/or disorder is heart failure.

[0197] In other embodiments, a method of treating heart failure in a patient, comprising administering a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide.

[0198] In other embodiments, a method of preventing or treating a cardiac disease or disorder in a subject, comprising administering a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide.

[0199] In yet other embodiments, a method of preventing or treating a cardiac disease or disorder in a subject, comprising administering a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide and one or more therapeutic agents prescribed by the medical caregiver. In embodiments, the at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide and one or more therapeutic agents prescribed by the medical caregiver are administered consecutively or at the same time.

Diagnostics, Therapeutics, Kits

[0200] The compositions herein and compounds of the present invention can be utilized for diagnostics, therapeutics, and prophylaxis, and as research reagents and components of kits.

[0201] The compositions disclosed herein are generally and variously useful for treatment of a subject having a cardiac disease or disorder, for example, heart failure or dilated cardiomyopathy. We may refer to a subject, patient, or individual interchangeably. A subject is effectively treated whenever a clinically beneficial result ensues. This may mean, for example, a complete resolution of the symptoms of a disease, a decrease in the severity of the symptoms of the disease, or a slowing of the disease's progression. These methods can further include the steps of a) identifying a subject (e.g., a patient and, more specifically, a human patient) who has a cardiac disease or disorder; and b) providing to the subject with a composition comprising a nucleic acid encoding a BAG3 polypeptide. The nucleic acid encoding the BAG3 polypeptide can be inserted into a vector, for example, an AAV vector, which is administered to the subject. A subject can be identified using standard clinical tests relating to cardiac function, for example. An amount of such a composition provided to the subject that results in a complete resolution of the symptoms of the infection, a decrease in the severity of the symptoms of the infection, or a slowing of the infection's progression is considered a therapeutically effective amount. The present methods may also include a monitoring step to help optimize dosing and scheduling as well as predict outcome. In some methods of the present invention, one can first determine whether a patient has decreased levels of BAG3 and then make a determination as to whether or not to treat the patient with one or more of the compositions described herein. BAG3 levels can be assayed using, for example, an anti-BAG3 antibody, and then compared to a reference level to determine whether the patient has elevated levels of BAG3. Monitoring can also be used to rapidly distinguish responsive patients from nonresponsive patients.

[0202] Cardiovascular disorders amenable to the therapeutic, and/or prognostic methods of the invention can be disorders that are responsive to the modulation of BAG3. While we believe we understand certain events that occur in the course of treatment, the compositions of the present invention are not limited to those that work by affecting any particular cellular mechanism. Any form of cardiovascular disorder which is associated with misregulation of BAGS is within the scope of the invention.

[0203] The methods of the invention can be expressed in terms of the preparation of a medicament. Accordingly, the invention encompasses the use of the agents and compositions described herein in the preparation of a medicament. The compounds described herein are useful in therapeutic compositions and regimens or for the manufacture of a medicament for use in treatment of diseases or conditions as described herein (e.g., a cardiovascular disorder disclosed herein).

[0204] Any composition described herein can be administered to any part of the host's body for subsequent delivery to a target cell. A composition can be delivered to, without limitation, the brain, the cerebrospinal fluid, joints, nasal mucosa, blood, lungs, intestines, muscle tissues, skin, or the peritoneal cavity of a mammal. In terms of routes of delivery, a composition can be administered by intravenous, intracranial, intraperitoneal, intramuscular, subcutaneous, intramuscular, intrarectal, intravaginal, intrathecal, intratracheal, intradermal, or transdermal injection, by oral or nasal administration, or by gradual perfusion over time. In a further example, an aerosol preparation of a composition can be given to a host by inhalation.

[0205] The dosage required will depend on the route of administration, the nature of the formulation, the nature of the patient's illness, the patient's size, weight, surface area, age, and sex, other drugs being administered, and the judgment of the attending clinicians. Suitable dosages are in the range of 0.01-1,000 mg/kg. Wide variations in the needed dosage are to be expected in view of the variety of cellular targets and the differing efficiencies of various routes of administration. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art. Administrations can be single or multiple (e.g., 2- or 3-, 4-, 6-, 8-, 10-, 20-, 50-, 100-, 150-, or more fold). Encapsulation of the compounds in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery.

[0206] The duration of treatment with any composition provided herein can be any length of time from as short as one day to as long as the life span of the host (e.g., many years). For example, a compound can be administered once a week (for, for example, 4 weeks to many months or years); once a month (for, for example, three to twelve months or for many years); or once a year for a period of 5 years, ten years, or longer. It is also noted that the frequency of treatment can be variable. For example, the present compounds can be administered once (or twice, three times, etc.) daily, weekly, monthly, or yearly.

[0207] An effective amount of any composition provided herein can be administered to an individual in need of treatment. The term "effective" as used herein refers to any amount that induces a desired response while not inducing significant toxicity in the patient. Such an amount can be determined by assessing a patient's response after administration of a known amount of a particular composition. In addition, the level of toxicity, if any, can be determined by assessing a patient's clinical symptoms before and after administering a known amount of a particular composition. It is noted that the effective amount of a particular composition administered to a patient can be adjusted according to a desired outcome as well as the patient's response and level of toxicity. Significant toxicity can vary for each particular patient and depends on multiple factors including, without limitation, the patient's disease state, age, and tolerance to side effects.

[0208] Any method known to those in the art can be used to determine if a particular response is induced. Clinical methods that can assess the degree of a particular disease state can be used to determine if a response is induced. The particular methods used to evaluate a response will depend upon the nature of the patient's disorder, the patient's age, and sex, other drugs being administered, and the judgment of the attending clinician.

[0209] Concurrent administration of two or more therapeutic agents does not require that the agents be administered at the same time or by the same route, as long as there is an overlap in the time period during which the agents are exerting their therapeutic effect. Simultaneous or sequential administration is contemplated, as is administration on different days or weeks. The compositions may also be administered with another standard therapeutic agent for treatment of cardiovascular disease.

[0210] In another preferred embodiment, the agents modulate the expression of Bcl-2 associated anthanogene-3 (BAG3) in patients suffering from or at risk of developing diseases or disorders associated with molecules or pathways associated with BAG3. Examples of such diseases or disorders associated comprise: cardiac diseases or disorders, skeletal muscle diseases or disorders, multiple sclerosis, senile plaques, cerebral amyloid angiopathy, atherosclerosis, glioblastoma, amyloid deposition, neurodegenerative diseases, neurofibrillary tangles, dementia, choriocarcinoma, astrocytoma, amyloidosis, hyperlipidemia, neurodegeneration, neoplastic transformation, prostate cancer, atherosclerotic plaque, obstruction, AIDS, metastasis, myocardial infarction, pulmonary fibrosis, necrosis, shock, melanoma, colorectal carcinoma, genetic susceptibility, psoriasis, cancer, inflammation, glioma, carcinoma, breast cancer, neuropathology, tumors, prostate carcinoma, vascular diseases, cell damage, brain tumors, Non-small cell lung carcinomas (NSCLCs), hypercholesterolemia. Examples of skeletal muscles diseases include, primary (genetic) diseases of muscle (e.g., muscular dystrophies and congenital myopathies, metabolic myopathies); acquired diseases (e.g. myositis, toxic myopathy); secondary diseases of muscle (e.g. neurogenic atrophy, atrophy from chronic pulmonary, heart, kidney disease, HIV/AIDs, cancer, sarcopenia and the like.

[0211] Kits:

[0212] The present invention further provides systems and kits (e.g., commercial therapeutic, diagnostic, or research products, reaction mixtures, etc.) that contain one or more or all components sufficient, necessary, or useful to practice any of the methods described herein. These systems and kits may include buffers, detection/imaging components, positive/negative control reagents, instructions, software, hardware, packaging, or other desired components.

[0213] The kits provide useful tools for screening test compounds capable of modulating the effects of a compound on a target molecule. The kits can be packaged in any suitable manner to aid research, clinical, and testing labs, typically with the various parts, in a suitable container along with instructions for use.

[0214] In certain embodiments, the kits may further comprise lipids and/or solvents. In certain embodiments, the kits may further comprise buffers and reagents needed for the procedure, and instructions for carrying out the assay. In certain embodiments, the kits may further comprise, where necessary, agents for reducing the background interference in a test, positive and negative control reagents, apparatus for conducting a test, and the like.

[0215] Also provided are kits for determining whether a subject has a mutation in a BAG3 polypeptide, for example, the 10 base pair deletion disclosed herein, to diagnose patients having cardiovascular disease or a predisposition to

developing cardiovascular disease. The kits can also be utilized to monitor the efficiency of agents used for treatment of cardiovascular disease.

Administration of Compositions

[0216] The agents identified by the methods embodied herein can be formulated and compositions of the present invention may be administered in conjunction with one or more additional active ingredients, pharmaceutical compositions, or other compounds. The therapeutic agents of the present invention may be administered to an animal, preferably a mammal, most preferably a human.

[0217] In some embodiments, a pharmaceutical composition comprises a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide is administered as part of the treatment.

[0218] In some embodiments, a pharmaceutical composition comprises a therapeutically effective amount of at least one agent which modulates, expression of BAG3, or a BAG3 polynucleotide or polypeptide and one or more therapeutic agents prescribed by the medical caregiver.

[0219] In other embodiments, a pharmaceutical composition comprises at least one or more candidate therapeutic agents embodied herein.

[0220] The pharmaceutical formulations may be for administration by oral (solid or liquid), parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), intracardial, transdermal (either passively or using ionophoresis or electroporation), transmucosal and systemic (nasal, vaginal, rectal, or sublingual), or inhalation routes of administration, or using bioerodible inserts and can be formulated in dosage forms appropriate for each route of administration.

[0221] The agents may be formulated in pharmaceutically acceptable carriers or diluents such as physiological saline or a buffered salt solution. Suitable carriers and diluents can be selected on the basis of mode and route of administration and standard pharmaceutical practice. A description of exemplary pharmaceutically acceptable carriers and diluents, as well as pharmaceutical formulations, can be found in Remington's Pharmaceutical Sciences, a standard text in this field, and in USP/NF. Other substances may be added to the compositions to stabilize and/or preserve the compositions.

[0222] The compositions of the invention may be administered to animals by any conventional technique. The compositions may be administered directly to a target site by, for example, surgical delivery to an internal or external target site, or by catheter to a site accessible by a blood vessel. Other methods of delivery, e.g., liposomal delivery or diffusion from a device impregnated with the composition, are known in the art. The compositions may be administered in a single bolus, multiple injections, or by continuous infusion (e.g., intravenously). For parenteral administration, the compositions are preferably formulated in a sterilized pyrogen-free form.

[0223] The compounds identified by this invention may also be administered orally to the patient, in a manner such that the concentration of drug is sufficient to inhibit bone resorption or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the condition of the patient. Preferably the oral dose would be about 0.5 to about 20 mg/kg.

[0224] An intravenous infusion of the compound in 5% dextrose in water or normal saline, or a similar formulation with suitable excipients, is most effective, although an intramuscular bolus injection is also useful. Typically, the parenteral dose will be about 0.01 to about 100 mg/kg; preferably between 0.1 and 20 mg/kg, in a manner to maintain the concentration of drug in the plasma at a concentration effective to increase BAGS expression. The compounds may be administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about 400 mg/kg/day. The precise amount of an inventive compound which is therapeutically effective, and the route by which such compound is best administered, is readily determined by one of ordinary skill in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect. Prodrugs of compounds of the present invention may be prepared by any suitable method.

[0225] No unacceptable toxicological effects are expected when compounds, derivatives, salts, compositions etc., of the present invention are administered in accordance with the present invention. The compounds of this invention, which may have good bioavailability, may be tested in one of several biological assays to determine the concentration of a compound which is required to have a given pharmacological effect.

[0226] In another preferred embodiment, there is provided a pharmaceutical or veterinary composition comprising one or more identified compounds and a pharmaceutically or veterinarily acceptable carrier. Other active materials may also be present, as may be considered appropriate or advisable for the disease or condition being treated or prevented.

[0227] The carrier, or, if more than one be present, each of the carriers, must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient.

[0228] The compounds identified by the methods herein would be suitable for use in a variety of drug delivery systems described above. Additionally, in order to enhance the in vivo serum half-life of the administered compound, the compounds may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional techniques may be employed which provide an extended serum half-life of the compounds. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka, et al., U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028 each of which is incorporated herein by reference. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody. The liposomes will be targeted to and taken up selectively by the organ.

[0229] The formulations include those suitable for rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration, but preferably the formulation is an orally administered formulation. The formulations may conveniently be presented in unit dosage form, e.g. tablets and sustained release capsules, and may be prepared by any methods well known in the art of pharmacy.

[0230] Such methods include the step of bringing into association the above defined active agent with the carrier. In general, the formulations are prepared by uniformly and intimately bringing into association the active agent with

liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

[0231] The compound identified using these methods can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the compound is combined in admixture with a pharmaceutically acceptable carrier vehicle. Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™. (ICI Americas Inc., Bridgewater, N.J.), PLURONICS™. (BASF Corporation, Mount Olive, N.J.) or PEG.

[0232] The formulations to be used for in vivo administration must be sterile and pyrogen free. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution.

[0233] Dosages and desired drug concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" In Toxicokinetics and New Drug Development, Yacobi et al., Eds., Pergamon Press, New York 1989, pp. 42-96.

[0234] Formulations for oral administration in the present invention may be presented as: discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active agent; as a powder or granules; as a solution or a suspension of the active agent in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water in oil liquid emulsion; or as a bolus etc.

[0235] For compositions for oral administration (e.g. tablets and capsules), the term "acceptable carrier" includes vehicles such as common excipients e.g. binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone (Povidone), methylcellulose, ethylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sucrose and starch; fillers and carriers, for example corn starch, gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid; and lubricants such as magnesium stearate, sodium stearate and other metallic stearates, glycerol stearate stearic acid, silicone fluid, talc waxes, oils and colloidal silica. Flavoring agents such as peppermint, oil of wintergreen, cherry flavoring and the like can also be used. It may be desirable to add a coloring agent to make the dosage form readily identifiable. Tablets may also be coated by methods well known in the art.

[0236] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active agent in a free flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active agent.

[0237] Other formulations suitable for oral administration include lozenges comprising the active agent in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active agent in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active agent in a suitable liquid carrier.

[0238] Parenteral formulations will generally be sterile.

[0239] Dose: An effective dose of a composition of the presently disclosed subject matter is administered to a subject in need thereof. A "therapeutically effective amount" or a "therapeutic amount" is an amount of a therapeutic composition sufficient to produce a measurable response (e.g., a biologically or clinically relevant response in a subject being treated). The response can be measured in many ways, as discussed above, e.g. cytokine profiles, cell types, cell surface molecules, etc. Actual dosage levels of active ingredients in the compositions of the presently disclosed subject matter can be varied so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular subject. The selected dosage level will depend upon the activity of the therapeutic composition, the route of administration, combination with other drugs or treatments, the severity of the condition being treated, and the condition and prior medical history of the subject being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. The potency of a composition can vary, and therefore a "treatment effective amount" can vary. However, using the assay methods described herein, one skilled in the art can readily assess the potency and efficacy of a candidate compound of the presently disclosed subject matter and adjust the therapeutic regimen accordingly.

[0240] The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modifications and improvements within the spirit and scope of the invention.

[0241] All documents mentioned herein are incorporated herein by reference. All publications and patent documents cited in this application are incorporated by reference for all purposes to the same extent as if each individual publication or patent document were so individually denoted. By their citation of various references in this document, Applicants do not admit any particular reference is "prior art" to their invention.

EXAMPLES

[0242] While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and not limitation. The following non-limiting examples are illustrative of the invention.

Example 1: Changes in BAG3 Protein Levels are Associated with Both Familial and Non-Familial Dilated Cardiomyopathy

[0243] Mutations in Bcl-2 associated anthanogene-3 (BAG3), a 575 amino acid anti-apoptotic protein that serves as a co-chaperone of the heat shock proteins (HSPs), has been associated with FDC (Selcen D, et al., *Annals of Neurology.* 2009; 65:83-89; Odgerel Z, et al., *Neuromuscular disorders: NMD.* 2010; 20:438-442; Lee H C, et al., *Clinical Genetics.* 2012; 81:394-398). For example, Norton et al. recently identified a deletion of BAG3 exon 4 as a rare variant causative of FDC in a family without neuropathy or peripheral muscle weakness (Norton N, et al., *American Journal of Human Genetics.* 2011; 88:273-282). Subsequent sequencing of BAG3 in subjects diagnosed with IDC identified four additional mutations that segregated with all relatives affected by the disease. A genome-wide association study conducted in patients with HF secondary to IDC implicated a non-synonymous single nucleotide polymorphism (SNP) (c.757T>C, [p. Cys151Arg]) located within the BAG3 gene as contributing to sporadic dilated cardiomyopathy (Villard E, et al., *European Heart Journal.* 2011; 32:1065-1076).

[0244] In the present study, a novel BAG3 mutation was identified in a family with adult-onset FDC. Furthermore, it is reported herein, for the first time, that BAG3 protein levels are significantly decreased in unrelated patients with non-familial IDC evidencing that altered levels of BAG3 protein may participate in the progression of HF.

[0245] Materials and Methods

[0246] Materials: A family with adult-onset familial dilated cardiomyopathy was identified. After obtaining informed consent, participating family members underwent a physical examination by a heart failure cardiologist and blood was collected for subsequent DNA analysis. DNA was extracted using a DNA extraction kit (Qiagen, Valencia Calif.) and stored at −70° C. Whenever possible, electrocardiograms were obtained from affected family members who had not undergone heart transplantation. Family members who had not had a recent echocardiogram underwent a transthoracic echocardiogram using a SonoHeart Elite (SonoSite Inc, Bothell, Wash., USA) portable echocardiographic system. Medical records were obtained from one individual who had died. Affection status was determined on the basis of consensus guidelines (Mestroni L, et al., *European Heart Journal.* 1999; 20:93-102). Participating family members provided written informed consent prior to evaluation and the protocols were approved by the Internal Review Boards of Thomas Jefferson University and of the University of Colorado.

[0247] Methods:

[0248] Human heart tissue was obtained from 9 subjects unrelated to the study family with end-stage heart failure undergoing heart transplant at Temple University Hospital (6 male, 3 female, mean age 47.6±5.7 years), from one affected family member at the time of heart transplantation at the University of Colorado and from 7 organ donors (1 male, 6 female, mean age 59.3±3.7 years) whose hearts were unsuitable for donation owing to blood type, age or size incompatibility. All of the patients undergoing transplantation had severe left ventricular dysfunction and cardiac dilation with a mean left ventricular ejection fraction (LVEF) of 12.8±1.4%. Two of the transplant recipients had HF secondary to ischemic cardiomyopathy and the remainder had non-ischemic IDC. Four of the transplant recipients were receiving dobutamine alone, 5 were receiving milrinone alone and one was receiving both milrinone and dobutamine at the time of the transplantation. Echocardiography was performed on all of the organ donors prior to organ donation and all had normal left ventricular function by echocardiography with a mean LVEF of 57.5±1.6%. Tissue aliquots were removed from the left ventricular free wall, rapidly frozen in liquid nitrogen and stored at −70° C. as described previously (Bristow M R, et al., *The Journal of Clinical Investigation.* 1993; 92:2737-2745). The Institutional Review Boards of the University of Colorado and Temple University approved the tissue study and consent was obtained for all subjects.

[0249] Exome Sequencing and Bioinformatics:

[0250] DNA from 5 affected family members and 1 unaffected family member was selected for exome sequencing with a target depth of >100×. Exome enrichment was performed using the Agilent SureSelect Human Exon 51 Mb kit (Agilent, Santa Clara, Calif.). Paired-end 100 nucleotide exome sequencing was performed using an Illumina HiSeq 2000 platform (San Diego, Calif.). Sequence reads passing Illumina chastity filter, were subjected to a quality filter step, trimmed and retained if the trimmed reads for each pair exceeded 50 nucleotides. Paired reads were then mapped to the reference human genome sequence (hg19) with gSNAP (Wu T D, et al., *Bioinformatics.* 2010; 26:873-881). Sequence calls for variants (single-nucleotide polymorphisms [SNPs)] insertions and deletions [indels]) were performed using the Broad's Genome Analysis Toolkit (McKenna A, et al., *Genome Research.* 2010; 20:1297-1303).

[0251] After variant detection, the program Annotate Variation (ANNOVAR) was used to classify variants (e.g., exonic, intronic, synonymous, non-synonymous, splice variant, stop gain, stop loss, insertion, or deletion) and to cross reference all the variants across various genetic variation databases (e.g., dbSNP, 1000 genomes database, AVSIFT) to isolate rare variants (variants with mean allele frequencies of <1% not found in dbSNP, 1000 genomes database, aVSIFT) (Wang K, et al., *Nucleic Acids Research.* 2010; 38:e164). Only non-synonymous changes (SNPs and in-dels), those that cause an alternate splice site, and/or an aberrant stop codon, were considered for further analysis. For non-synonymous changes, all insertion and deletion variants were considered damaging, whereas SNP variants were cross-referenced to the dbNSFP database to determine whether the changes to the protein structure would be considered tolerable or damaging using four algorithms (Sorting Intolerant From Tolerant (SIFT), PolyPhen2, likelihood ratio test [LRT], or MutationTaster) (Liu X, et al., Human Mutation. 2011; 32:894-899). Putative mutations identified were confirmed with traditional Sanger sequencing in both affected and unaffected family members (primers and conditions available upon request).

[0252] Western Blot Analysis of Human Heart Tissue:

[0253] Frozen tissue was homogenized in 40 mM Tris buffer, pH 7.5 containing 150 mM NaCl, 1% NP40, 1 mM DTT, and 1 mM EDTA. The sample was then centrifuged at 10,000×g at 4° C. for 30 min and the supernatant was collected and re-suspended in 350 uM Tris buffer, pH 6.8 containing 25% beta-mercaptoethanol, 30% glycerol, 10% SDS, and 2% bromophenol blue. The protein concentration was measured using the method of Bradford and the samples were stored at −80° C. Equal amounts of protein (10 μg) were fractionated by SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membrane. Membranes were blocked in 10% nonfat dry milk/tris-buffered saline (pH 7.6) plus 0.1% Tween-20 (TBS-T) for 1 h and then incubated with polyclonal BAG3 antibody (Proteintech, Chicago, Ill.) in 5% nonfat dry milk with PBST for 2 hrs. Membranes were then incubated with goat-anti-rabbit 800 and goat-anti-mouse secondary antibody for 1 hr and scanned on a LI-COR Odyssey imaging system (Lincoln Nebr.). All Western blot procedures were carried out at room temperature. BAG3 signal intensity was normalized to GAPDH.

[0254] Results

[0255] Family History:

[0256] The proband (FIG. **1**, III-5) was a 65 year-old woman of Eastern European ancestry who was referred in June 2003 to the heart failure clinic at Thomas Jefferson University because of a family history of HF. She had first been noted to have a dilated cardiomyopathy at 45 years of age. She was largely asymptomatic while receiving a diuretic, a β-adrenergic receptor antagonist (β-blocker) and an angiotensin converting enzyme (ACE) inhibitor. Her vital signs were within normal limits and her physical examination was notable only for a soft S3 heart sound. She had no peripheral muscle weakness and her neurologic examination was unremarkable. Her electrocardiogram revealed normal sinus rhythm with mild LV hypertrophy and non-specific ST-T wave changes. Her left ventricular ejection fraction was 20% by echocardiography. As seen in FIG. **1** and Table 1, the proband had two female siblings, one of whom (III-7) was asymptomatic with a normal physical examination; however, her ejection fraction by echocardiography was 44%. A second sister (III-9) was phenotypically normal and had a normal echocardiogram.

[0257] The proband had three children. A son underwent cardiac transplantation at the age of 20 secondary to IDC (IV-5), a second son was diagnosed with idiopathic dilated cardiomyopathy at the age of 20 but remained asymptomatic at age 32 despite an ejection fraction of 33% (IV-4). A daughter had no cardiac symptoms; however, her left ventricular ejection fraction by echocardiography was 48% and she had mild dilatation of the left ventricle and the aortic root without obvious aortic valve disease. (IV-6) Her echocardiogram met the criteria for diagnosis of a dilated cardiomyopathy. Her electrocardiogram was normal. Neurologic function was normal in all three children. The proband's affected sister (III-7) had one daughter who died of progressive heart failure secondary to IDC at the age of 22. (IV-7): two other children had normal echocardiograms. A cousin underwent cardiac transplantation because of IDC at 42 years of age after diagnosis at the age of 40 (III-1) and one of his sons also underwent cardiac transplantation for IDC at the University of Colorado at the age of 30 (IV-1). Healthy subjects were defined as "non-affected" if they had reached the age of 40 without symptoms and had a normal echocardiogram that did not meet the criteria for diagnosis of a cardiomyopathy. Ten-year follow-up of all participants demonstrated that functional capacity had remained stable in all family members.

[0258] Genetic Analysis:

[0259] As seen in FIG. **1**, the pedigree and clinical data were compatible with autosomal dominant adult-onset familial IDC. Exome sequencing of the DNA from 5 affected (III-5, 7: IV-1, 4, 5) and 1 unaffected (III-9) family members had an average of 11.8±0.96 Gb of past-filter sequence reads per sample. After bioinformatics filtering a 10-nucleotide deletion in the coding portion of exon 4 of BAG3 (Ch10:del 121436332_12143641: del. 1266_1275 [NM 004281]) was noted to be present in all tested affected subjects and absent in the one healthy sister of the proband (III-9) (FIG. **2**). Additional family members were tested for the BAG3 deletion by Sanger sequencing confirming appropriate co-segregation of the deletion with the phenotype among affected (III-1, 5, 7 and IV-1, 4, 5, 6) and unaffected (III-9 and IV-8, 9, 10, 11, 12) individuals. This deletion was not found in existing databases and introduces a frame shift and premature stop codon after 13 amino acids that predicts truncation of BAG3 at the carboxy terminal end by 140 amino acids. Thus, the abnormal BAG3 protein is predicted to have 435 amino acids instead of 575 amino acids. In addition, the amino acid sequence distal to the deletion (K P S W R R Y R G W S R L) (SEQ ID NO:8) is predicted to be different from that found in the normal protein. Only one additional variant was found by exome sequencing and after bioinformatics filtering. The variant (rs8192669), found in the IKZF5 gene did not segregate according to the IDC phenotype in other family members. An analysis of 52 genes previously associated with monogenic IDC for rare variants (≤1%) identified only non-synonymous mutations in TTN, GATAD1, MYPN, ANKRD1 and RBM20: none of these variants segregated with the disease phenotype.

[0260] BAG 3 Expression in Failing Human Heart:

[0261] In order to determine whether the BAG3 deletion (BAG3 del. NM_004281) found in this patient cohort resulted in a decrease in the levels of BAG3 protein, Western blot analysis was performed on cardiac muscle obtained from one affected family member (IV-1) who underwent cardiac transplantation. The level of BAG3 protein in subject IV-1 was less than half that seen in heart tissue obtained from organ donors whose heart could not be utilized for transplantation. As seen in FIGS. **3**A and **3**B, BAG3 levels in failing human heart from patients with end stage heart failure without known BAG3 mutations were significantly ($p=0.0002$) less than that found in non-failing control hearts. Thus it appears that decreased levels of BAG3 protein can be found both in individuals with a BAG3 mutation as well as in end-stage failing human heart.

DISCUSSION

[0262] It is being increasingly recognized that genetic mutations can account for as many as a third of cases of IDC. Indeed, investigators have begun to refer to these cases as familial dilated cardiomyopathy (FDC). Inheritance can occur in a variety of manners with the most common pattern of inheritance being autosomal dominant. Mutations are most commonly found in genes encoding the sarcomere leading to cardiac dysfunction, disintegration of the myofiber structure and accumulation of degraded material in

autophagic granules. Here, it is reported that a 10 bp deletion in the gene encoding the sarcomeric protein BAG3 segregates completely with affected individuals in a family with an autosomal dominant pattern of FDC. It is also report for the first time that BAG3 protein is substantially reduced in the hearts of unrelated patients who are undergoing heart transplantation when compared with normal hearts from transplant recipients.

[0263] BAG3 is a 575 amino acid anti-apoptotic protein that is constitutively expressed in the heart and serves as a co-chaperone of the heat shock proteins (HSPs). BAG3 binds to HSPs and regulates their ability to chaperone cytoskeletal proteins including desmin and also participate in degradation of cellular proteins through either the proteasome or autophagy pathways. BAG3 also protects cells from apoptotic death and inhibits myofibrillar degeneration in response to mechanical stress. Knockdown of BAG3 in zebrafish or in neonatal cardiomyoctes or homozygous disruption of BAG3 in mice leads to cardiac dysfunction and BAG3 levels are decreased in the skeletal muscle of spontaneously hypertensive rats.

[0264] The results of the present study in a large family with FDC are consistent with earlier reports that demonstrated an association between mutations in BAG3 and the development of muscle pathology. Mutations in BAG3 were first shown to cause abnormal muscle function in two families with childhood-onset muscular dystrophy (Selcen D, et al., *Annals of Neurology.* 2009; 65:83-89; Odgerel Z, et al., Neuromuscular Disorders: NMD. 2010; 20:438-442) and the phenotype of IDC, diffuse myocardial fibrosis and sudden death was linked with markers in the chromosome 10q25-26 region which includes the BAG3 locus. More recent studies have demonstrated a causative relationship between BAG3 mutations and the development of FDC without peripheral muscle weakness or neurologic findings (Norton N, et al., *American Journal of Human Genetics.* 2011; 88:273-282; Villard E, et al., *European Heart Journal.* 2011; 32:1065-1076; Arimura T, et al., *Human Mutation.* 2011; 32:1481-1491).

[0265] As seen with genetic variants in other sarcomeric genes, there was substantial genetic heterogeneity within this large family. For example, one of the proband's sons had an early onset of severe disease requiring transplantation whereas a sibling with moderate disease and a middle-aged daughter with very mild disease remain asymptomatic for over a decade. Indeed, the cardiac dysfunction in the proband's daughter would have gone unrecognized had it not been for careful phenotyping as part of this study. Identification of the causative mutation in this family provides an opportunity for guideline-driven genetic testing and counseling of family members and early identification of affected individuals. The finding that use of an angiotensin converting enzyme inhibitor improved survival in a small group of patients with Duchenne muscular dystrophy suggests that early therapy in families with mutations in sarcomere genes might be beneficial; however, additional studies will be required to define the best treatment strategies.

[0266] It is reported herein, for the first time that the level of BAG3 protein is significantly reduced in the hearts of unrelated patients with end-stage HF who are undergoing heart transplant and who have no family history of heart muscle disease. This finding is interesting as it evidences that while mutations in BAG3 can be causative of disease in FDC, changes in levels of BAG3 protein may participate in the progression of disease in patients with non-familial forms of IDC. Nonetheless, these results evidence that BAG3 protein might be a new target for therapeutic intervention in HF.

Example 2: Changes in BAG3 Protein Levels in Failing Murine Hearts

[0267] Wild type c57BL/6 mice underwent trans-aortic banding (TAC) as described in Tilley et al. (*Circulation* 2014, Nov. 11; 130(20):1800-11). Eighteen weeks after TAC, left ventricular contractility was measured using a conductance catheter inserted into the left ventricle through a carotid approach as described previously. Contractility was measured during intravenous infusion of increasing doses of catecholamine. (FIG. **6**B) Heart weight to body weight ratios were calculated after sacrifice. (FIG. **6**A). Hearts were then frozen for subsequent measurement of BAG3 levels. Myocardial proteins were extracted as described in Example 1, separated by gel electrophoresis and probed with a murine BAG3 antibody. As shown in FIG. **6**C, there was a significant decrease in BAG3 levels by Western blotting in TAC mice when compared with sham-operated controls. A representative Western blot is shown in FIG. **6**D.

Example 3: Changes in BAG3 Protein Levels in Porcine Hearts Following Balloon Occlusion

[0268] Hemodynamic indices and BAG3 levels were measured in non-infarcted left ventricular myocardium from a pig 4 weeks after balloon occlusion of the left anterior descending coronary artery. As shown in **7**A, **7**B, **7**C, and **7**D, ejection fraction, fractional shortening, end-diastolic volume, and end systolic volume, respectively, were significantly altered following balloon occlusion. As shown graphically in FIG. **7**E, and in the Western blot in FIG. **7**F, BAG3 levels were reduced in porcine hearts following balloon occlusion.

TABLE 1

Phenotype of study subjects with and without a 10-nucleotide deletion in the BAG3 gene.

Subject	Age Eval/Onset/Death or Transpl	Gender	EF(%)	ECG	Mutation	Comment
II-1	na/na/70+	M				Died late 70's, hx of HF
II-3	na/na/80	F				Hx of HBP and CVA
II-4	na/na/29	M				motor vehicle accident
III-1	62/40/42	M			Yes	transplant at 42
III-5	65/45/na	F	20	NS-ST-T changes	Yes	

TABLE 1-continued

Phenotype of study subjects with and without a 10-nucleotide deletion in the BAG3 gene.

Subject	Age Eval/Onset/Death or Transpl	Gender	EF(%)	ECG	Mutation	Comment
III-7	67/47/na	F	44	nl	Yes	asymptomatic
III-9	68/na/na	F	58	nl	No	
IV-1	30/30/30	M			Yes	transplant at 30
IV-4	39/20/na	M	33	sinus brady, IVCD	Yes	asymptomatic
IV-5	35/20/20	M			Yes	transplant at 20
IV-6	34/34/na	F	48	nl	Yes	mild aortic root dilat, LVDD 5.8
IV-7	na/18/22	F				died-worsening HF
IV-8	38	F	nl		No	
IV-9	42	M	nl		No	
IV-10	41	F	nl		No	
IV-11	44	M	nl		No	
IV-12	45	M	nl		No	

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 51

<210> SEQ ID NO 1
<211> LENGTH: 575
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

Met Ser Ala Ala Thr His Ser Pro Met Met Gln Val Ala Ser Gly Asn
1 5 10 15

Gly Asp Arg Asp Pro Leu Pro Pro Gly Trp Glu Ile Lys Ile Asp Pro
20 25 30

Gln Thr Gly Trp Pro Phe Phe Val Asp His Asn Ser Arg Thr Thr Thr
35 40 45

Trp Asn Asp Pro Arg Val Pro Ser Glu Gly Pro Lys Glu Thr Pro Ser
50 55 60

Ser Ala Asn Gly Pro Ser Arg Glu Gly Ser Arg Leu Pro Pro Ala Arg
65 70 75 80

Glu Gly His Pro Val Tyr Pro Gln Leu Arg Pro Gly Tyr Ile Pro Ile
85 90 95

Pro Val Leu His Glu Gly Ala Glu Asn Arg Gln Val His Pro Phe His
100 105 110

Val Tyr Pro Gln Pro Gly Met Gln Arg Phe Arg Thr Glu Ala Ala Ala
115 120 125

Ala Ala Pro Gln Arg Ser Gln Ser Pro Leu Arg Gly Met Pro Glu Thr
130 135 140

Thr Gln Pro Asp Lys Gln Cys Gly Gln Val Ala Ala Ala Ala Ala Ala
145 150 155 160

Gln Pro Pro Ala Ser His Gly Pro Glu Arg Ser Gln Ser Pro Ala Ala
165 170 175

Ser Asp Cys Ser Ser Ser Ser Ser Ser Ala Ser Leu Pro Ser Ser Gly
180 185 190

Arg Ser Ser Leu Gly Ser His Gln Leu Pro Arg Gly Tyr Ile Ser Ile
195 200 205

Pro Val Ile His Glu Gln Asn Val Thr Arg Pro Ala Ala Gln Pro Ser
210 215 220

-continued

Phe His Gln Ala Gln Lys Thr His Tyr Pro Ala Gln Gln Gly Glu Tyr
225 230 235 240

Gln Thr His Gln Pro Val Tyr His Lys Ile Gln Gly Asp Asp Trp Glu
245 250 255

Pro Arg Pro Leu Arg Ala Ala Ser Pro Phe Arg Ser Ser Val Gln Gly
260 265 270

Ala Ser Ser Arg Glu Gly Ser Pro Ala Arg Ser Ser Thr Pro Leu His
275 280 285

Ser Pro Ser Pro Ile Arg Val His Thr Val Val Asp Arg Pro Gln Gln
290 295 300

Pro Met Thr His Arg Glu Thr Ala Pro Val Ser Gln Pro Glu Asn Lys
305 310 315 320

Pro Glu Ser Lys Pro Gly Pro Val Gly Pro Glu Leu Pro Pro Gly His
325 330 335

Ile Pro Ile Gln Val Ile Arg Lys Glu Val Asp Ser Lys Pro Val Ser
340 345 350

Gln Lys Pro Pro Pro Pro Ser Glu Lys Val Glu Val Lys Val Pro Pro
355 360 365

Ala Pro Val Pro Cys Pro Pro Pro Ser Pro Gly Pro Ser Ala Val Pro
370 375 380

Ser Ser Pro Lys Ser Val Ala Thr Glu Glu Arg Ala Ala Pro Ser Thr
385 390 395 400

Ala Pro Ala Glu Ala Thr Pro Pro Lys Pro Gly Glu Ala Glu Ala Pro
405 410 415

Pro Lys His Pro Gly Val Leu Lys Val Glu Ala Ile Leu Glu Lys Val
420 425 430

Gln Gly Leu Glu Gln Ala Val Asp Asn Phe Glu Gly Lys Lys Thr Asp
435 440 445

Lys Lys Tyr Leu Met Ile Glu Glu Tyr Leu Thr Lys Glu Leu Leu Ala
450 455 460

Leu Asp Ser Val Asp Pro Glu Gly Arg Ala Asp Val Arg Gln Ala Arg
465 470 475 480

Arg Asp Gly Val Arg Lys Val Gln Thr Ile Leu Glu Lys Leu Glu Gln
485 490 495

Lys Ala Ile Asp Val Pro Gly Gln Val Gln Val Tyr Glu Leu Gln Pro
500 505 510

Ser Asn Leu Glu Ala Asp Gln Pro Leu Gln Ala Ile Met Glu Met Gly
515 520 525

Ala Val Ala Ala Asp Lys Gly Lys Lys Asn Ala Gly Asn Ala Glu Asp
530 535 540

Pro His Thr Glu Thr Gln Gln Pro Glu Ala Thr Ala Ala Ala Thr Ser
545 550 555 560

Asn Pro Ser Ser Met Thr Asp Thr Pro Gly Asn Pro Ala Ala Pro
565 570 575

<210> SEQ ID NO 2
<211> LENGTH: 2608
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

gcggagctcc gcatccaacc ccgggccgcg gccaacttct ctggactgga ccagaagttt 60

-continued

ctagccggcc agttgctacc tccctttatc tcctccttcc cctctggcag cgaggaggct	120
atttccagac acttccaccc ctctctggcc acgtcacccc cgcctttaat tcataaaggt	180
gcccggcgcc ggcttcccgg acacgtcggc ggcggagagg ggcccacggc ggcggcccgg	240
ccagagactc ggcgcccgga gccagcgccc cgcacccgcg ccccagcggg cagaccccaa	300
cccagcatga gcgccgccac ccactcgccc atgatgcagg tggcgtccgg caacggtgac	360
cgcgaccctt tgcccccccgg atgggagatc aagatcgacc cgcagaccgg ctggcccttc	420
ttcgtggacc acaacagccg caccactacg tggaacgacc cgcgcgtgcc ctctgagggc	480
cccaaggaga ctccatcctc tgccaatggc ccttcccggg agggctctag gctgccgcct	540
gctagggaag gccaccctgt gtacccccag ctccgaccag gctacattcc cattcctgtg	600
ctccatgaag gcgctgagaa ccggcaggtg cacccctttcc atgtctatcc ccagcctggg	660
atgcagcgat tccgaactga ggcggcagca gcggctcctc agaggtccca gtcacctctg	720
cggggcatgc cagaaaccac tcagccagat aaacagtgtg gacaggtggc agcggcggcg	780
gcagcccagc ccccagcctc ccacggacct gagcggtccc agtctccagc tgcctctgac	840
tgctcatcct catcctcctc ggccagcctg ccttcctccg gcaggagcag cctgggcagt	900
caccagctcc cgcgggggta catctccatt ccggtgatac acgagcagaa cgttacccgg	960
ccagcagccc agccctcctt ccaccaagcc cagaagacgc actacccagc gcagcagggg	1020
gagtaccaga cccaccagcc tgtgtaccac aagatccagg gggatgactg ggagccccgg	1080
cccctgcggg cggcatcccc gttcaggtca tctgtccagg gtgcatcgag ccgggagggc	1140
tcaccagcca ggagcagcac gccactccac tccccctcgc ccatccgtgt gcacaccgtg	1200
gtcgacaggc ctcagcagcc catgacccat cgagaaactg cacctgtttc ccagcctgaa	1260
aacaaaccag aaagtaagcc aggcccagtt ggaccagaac tccctcctgg acacatccca	1320
attcaagtga tccgcaaaga ggtggattct aaacctgttt cccagaagcc cccacctccc	1380
tctgagaagg tagaggtgaa agttcccccc gctccagttc cttgtcctcc tcccagccct	1440
ggcccttctg ctgtcccctc ttccccaag agtgtggcta cagaagagag ggcagccccc	1500
agcactgccc ctgcagaagc tacacctcca aaaccaggag aagccgaggc tccccaaaa	1560
catccaggag tgctgaaagt ggaagccatc ctggagaagg tacaggggct ggagcaggct	1620
gtagacaact ttgaaggcaa gaagactgac aaaaagtacc tgatgatcga agagtatttg	1680
accaaagagc tgctggccct ggattcagtg gaccccgagg gacgagccga tgtgcgtcag	1740
gccaggagag acggtgtcag gaaggttcag accatcttgg aaaaacttga acagaaagcc	1800
attgatgtcc caggtcaagt ccaggtctat gaactccagc ccagcaacct tgaagcagat	1860
cagccactgc aggcaatcat ggagatgggt gccgtggcag cagacaaggg caagaaaaat	1920
gctggaaatg cagaagatcc ccacacagaa acccagcagc cagaagccac agcagcagcg	1980
acttcaaacc ccagcagcat gacagacacc cctggtaacc cagcagcacc gtagcctctg	2040
ccctgtaaaa atcagactcg gaaccgatgt gtgctttagg gaattttaag ttgcatgcat	2100
ttcagagact ttaagtcagt tggtttttat tagctgcttg gtatgcagta acttgggtgg	2160
aggcaaaaca ctaataaaag ggctaaaaag gaaaatgatg cttttcttct atattcttac	2220
tctgtacaaa taaagaagtt gcttgttgtt tgagaagttt aaccccgttg cttgttgttc	2280
tgcagccctg tctacttggg caccccccacc acctgttagc tgtggttgtg cactgtcttt	2340

-continued

tgtagctctg gactggaggg gtagatgggg agtcaattac ccatcacata aatatgaaac 2400

atttatcaga aatgttgcca ttttaatgag atgatttttct tcatctcata attaaaatac 2460

ctgactttag agagagtaaa atgtgccagg agccatagga atatctgtat gttggatgac 2520

tttaatgcta cattttaaaa aaagaaaata aagtaataat ataactcaaa aaaaaaaaaa 2580

aaaaaaaaaa aaaaaaaaaa aaaaaaaa 2608

<210> SEQ ID NO 3
<211> LENGTH: 575
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Ser Ala Ala Thr His Ser Pro Met Met Gln Val Ala Ser Gly Asn
1 5 10 15

Gly Asp Arg Asp Pro Leu Pro Pro Gly Trp Glu Ile Lys Ile Asp Pro
20 25 30

Gln Thr Gly Trp Pro Phe Phe Val Asp His Asn Ser Arg Thr Thr Thr
35 40 45

Trp Asn Asp Pro Arg Val Pro Ser Glu Gly Pro Lys Glu Thr Pro Ser
50 55 60

Ser Ala Asn Gly Pro Ser Arg Glu Gly Ser Arg Leu Pro Pro Ala Arg
65 70 75 80

Glu Gly His Pro Val Tyr Pro Gln Leu Arg Pro Gly Tyr Ile Pro Ile
85 90 95

Pro Val Leu His Glu Gly Ala Glu Asn Arg Gln Val His Pro Phe His
100 105 110

Val Tyr Pro Gln Pro Gly Met Gln Arg Phe Arg Thr Glu Ala Ala Ala
115 120 125

Ala Ala Pro Gln Arg Ser Gln Ser Pro Leu Arg Gly Met Pro Glu Thr
130 135 140

Thr Gln Pro Asp Lys Gln Cys Gly Gln Val Ala Ala Ala Ala Ala Ala
145 150 155 160

Gln Pro Pro Ala Ser His Gly Pro Glu Arg Ser Gln Ser Pro Ala Ala
165 170 175

Ser Asp Cys Ser Ser Ser Ser Ser Ser Ala Ser Leu Pro Ser Ser Gly
180 185 190

Arg Ser Ser Leu Gly Ser His Gln Leu Pro Arg Gly Tyr Ile Ser Ile
195 200 205

Pro Val Ile His Glu Gln Asn Val Thr Arg Pro Ala Ala Gln Pro Ser
210 215 220

Phe His Gln Ala Gln Lys Thr His Tyr Pro Ala Gln Gln Gly Glu Tyr
225 230 235 240

Gln Thr His Gln Pro Val Tyr His Lys Ile Gln Gly Asp Asp Trp Glu
245 250 255

Pro Arg Pro Leu Arg Ala Ala Ser Pro Phe Arg Ser Ser Val Gln Gly
260 265 270

Ala Ser Ser Arg Glu Gly Ser Pro Ala Arg Ser Ser Thr Pro Leu His
275 280 285

Ser Pro Ser Pro Ile Arg Val His Thr Val Val Asp Arg Pro Gln Gln
290 295 300

Pro Met Thr His Arg Glu Thr Ala Pro Val Ser Gln Pro Glu Asn Lys

-continued

305 310 315 320

Pro Glu Ser Lys Pro Gly Pro Val Gly Pro Glu Leu Pro Pro Gly His
325 330 335

Ile Pro Ile Gln Val Ile Arg Lys Glu Val Asp Ser Lys Pro Val Ser
340 345 350

Gln Lys Pro Pro Pro Pro Ser Glu Lys Val Glu Val Lys Val Pro Pro
355 360 365

Ala Pro Val Pro Cys Pro Pro Pro Ser Pro Gly Pro Ser Ala Val Pro
370 375 380

Ser Ser Pro Lys Ser Val Ala Thr Glu Glu Arg Ala Ala Pro Ser Thr
385 390 395 400

Ala Pro Ala Glu Ala Thr Pro Pro Lys Pro Gly Glu Ala Glu Ala Pro
405 410 415

Pro Lys His Pro Gly Val Leu Lys Val Glu Ala Ile Leu Glu Lys Val
420 425 430

Gln Gly Leu Glu Gln Ala Val Asp Asn Phe Glu Gly Lys Lys Thr Asp
435 440 445

Lys Lys Tyr Leu Met Ile Glu Glu Tyr Leu Thr Lys Glu Leu Leu Ala
450 455 460

Leu Asp Ser Val Asp Pro Glu Gly Arg Ala Asp Val Arg Gln Ala Arg
465 470 475 480

Arg Asp Gly Val Arg Lys Val Gln Thr Ile Leu Glu Lys Leu Glu Gln
485 490 495

Lys Ala Ile Asp Val Pro Gly Gln Val Gln Val Tyr Glu Leu Gln Pro
500 505 510

Ser Asn Leu Glu Ala Asp Gln Pro Leu Gln Ala Ile Met Glu Met Gly
515 520 525

Ala Val Ala Ala Asp Lys Gly Lys Lys Asn Ala Gly Asn Ala Glu Asp
530 535 540

Pro His Thr Glu Thr Gln Gln Pro Glu Ala Thr Ala Ala Ala Thr Ser
545 550 555 560

Asn Pro Ser Ser Met Thr Asp Thr Pro Gly Asn Pro Ala Ala Pro
565 570 575

<210> SEQ ID NO 4
<211> LENGTH: 575
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Ser Ala Ala Thr His Ser Pro Met Met Gln Val Ala Ser Gly Asn
1 5 10 15

Gly Asp Arg Asp Pro Leu Pro Pro Gly Trp Glu Ile Lys Ile Asp Pro
20 25 30

Gln Thr Gly Trp Pro Phe Phe Val Asp His Asn Ser Arg Thr Thr Thr
35 40 45

Trp Asn Asp Pro Arg Val Pro Ser Glu Gly Pro Lys Glu Thr Pro Ser
50 55 60

Ser Ala Asn Gly Pro Ser Arg Glu Gly Ser Arg Leu Pro Pro Ala Arg
65 70 75 80

Glu Gly His Pro Val Tyr Pro Gln Leu Arg Pro Gly Tyr Ile Pro Ile
85 90 95

-continued

Pro Val Leu His Glu Gly Ala Glu Asn Arg Gln Val His Pro Phe His
100 105 110

Val Tyr Pro Gln Pro Gly Met Gln Arg Phe Arg Thr Glu Ala Ala Ala
115 120 125

Ala Ala Pro Gln Arg Ser Gln Ser Pro Leu Arg Gly Met Pro Glu Thr
130 135 140

Thr Gln Pro Asp Lys Gln Cys Gly Gln Val Ala Ala Ala Ala Ala Ala
145 150 155 160

Gln Pro Pro Ala Ser His Gly Pro Glu Arg Ser Gln Ser Pro Ala Ala
165 170 175

Ser Asp Cys Ser Ser Ser Ser Ser Ser Ala Ser Leu Pro Ser Ser Gly
180 185 190

Arg Ser Ser Leu Gly Ser His Gln Leu Pro Arg Gly Tyr Ile Ser Ile
195 200 205

Pro Val Ile His Glu Gln Asn Val Thr Arg Pro Ala Ala Gln Pro Ser
210 215 220

Phe His Gln Ala Gln Lys Thr His Tyr Pro Ala Gln Gln Gly Glu Tyr
225 230 235 240

Gln Thr His Gln Pro Val Tyr His Lys Ile Gln Gly Asp Asp Trp Glu
245 250 255

Pro Arg Pro Leu Arg Ala Ala Ser Pro Phe Arg Ser Ser Val Gln Gly
260 265 270

Ala Ser Ser Arg Glu Gly Ser Pro Ala Arg Ser Ser Thr Pro Leu His
275 280 285

Ser Pro Ser Pro Ile Arg Val His Thr Val Val Asp Arg Pro Gln Gln
290 295 300

Pro Met Thr His Arg Glu Thr Ala Pro Val Ser Gln Pro Glu Asn Lys
305 310 315 320

Pro Glu Ser Lys Pro Gly Pro Val Gly Pro Glu Leu Pro Pro Gly His
325 330 335

Ile Pro Ile Gln Val Ile Arg Lys Glu Val Asp Ser Lys Pro Val Ser
340 345 350

Gln Lys Pro Pro Pro Pro Ser Glu Lys Val Glu Val Lys Val Pro Pro
355 360 365

Ala Pro Val Pro Cys Pro Pro Pro Ser Pro Gly Pro Ser Ala Val Pro
370 375 380

Ser Ser Pro Lys Ser Val Ala Thr Glu Glu Arg Ala Ala Pro Ser Thr
385 390 395 400

Ala Pro Ala Glu Ala Thr Leu Pro Lys Pro Gly Glu Ala Glu Ala Pro
405 410 415

Pro Lys His Pro Gly Val Leu Lys Val Glu Ala Ile Leu Glu Lys Val
420 425 430

Gln Gly Leu Glu Gln Ala Val Asp Asn Phe Glu Gly Lys Lys Thr Asp
435 440 445

Lys Lys Tyr Leu Met Ile Glu Glu Tyr Leu Thr Lys Glu Leu Leu Ala
450 455 460

Leu Asp Ser Val Asp Pro Glu Gly Arg Ala Asp Val Arg Gln Ala Arg
465 470 475 480

Arg Asp Gly Val Arg Lys Val Gln Thr Ile Leu Glu Lys Leu Glu Gln
485 490 495

Lys Ala Ile Asp Val Pro Gly Gln Val Gln Val Tyr Glu Leu Gln Pro

-continued

```
            500                 505                 510

Ser Asn Leu Glu Ala Asp Gln Pro Leu Gln Ala Ile Met Glu Met Gly
        515                 520                 525

Ala Val Ala Ala Asp Lys Gly Lys Lys Asn Ala Gly Asn Ala Glu Asp
    530                 535                 540

Pro His Thr Glu Thr Gln Gln Pro Glu Ala Thr Ala Ala Ala Thr Ser
545                 550                 555                 560

Asn Pro Ser Ser Met Thr Asp Thr Pro Gly Asn Pro Ala Ala Pro
                565                 570                 575


<210> SEQ ID NO 5
<211> LENGTH: 574
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Met Ser Ala Ala Thr His Ser Pro Met Met Gln Val Ala Ser Gly Asn
1               5                   10                  15

Gly Asp Arg Asp Pro Leu Pro Pro Gly Trp Glu Ile Lys Ile Asp Pro
            20                  25                  30

Gln Thr Gly Trp Pro Phe Phe Val Asp His Asn Ser Arg Thr Thr Thr
        35                  40                  45

Trp Asn Asp Pro Arg Val Pro Ser Glu Gly Pro Lys Glu Thr Pro Ser
    50                  55                  60

Ser Ala Asn Gly Pro Ser Arg Glu Gly Ser Arg Leu Pro Pro Ala Arg
65                  70                  75                  80

Glu Gly His Pro Val Tyr Pro Gln Leu Arg Pro Gly Tyr Ile Pro Ile
                85                  90                  95

Pro Val Leu His Glu Gly Ala Glu Asn Arg Gln Val His Pro Phe His
            100                 105                 110

Val Tyr Pro Gln Pro Gly Met Gln Arg Phe Arg Thr Glu Ala Ala Ala
        115                 120                 125

Ala Ala Pro Gln Arg Ser Gln Ser Pro Leu Arg Gly Met Pro Glu Thr
    130                 135                 140

Thr Gln Pro Asp Lys Gln Cys Gly Gln Val Ala Ala Ala Ala Ala Ala
145                 150                 155                 160

Gln Pro Pro Ala Ser His Gly Pro Glu Arg Ser Gln Ser Pro Ala Ala
                165                 170                 175

Ser Asp Cys Ser Ser Ser Ser Ser Ser Ala Ser Leu Pro Ser Ser Gly
            180                 185                 190

Arg Ser Ser Leu Gly Ser His Gln Leu Pro Arg Gly Tyr Ile Ser Ile
        195                 200                 205

Pro Val Ile His Glu Gln Asn Val Thr Arg Pro Ala Ala Gln Pro Ser
    210                 215                 220

Phe His Gln Ala Gln Lys Thr His Tyr Pro Ala Gln Gln Gly Glu Tyr
225                 230                 235                 240

Gln Thr His Gln Pro Val Tyr His Lys Ile Gln Gly Asp Asp Trp Glu
                245                 250                 255

Pro Arg Pro Leu Arg Ala Ala Ser Pro Phe Arg Ser Ser Val Gln Gly
            260                 265                 270

Ala Ser Ser Arg Glu Gly Ser Pro Ala Arg Ser Ser Thr Pro Leu His
        275                 280                 285
```

-continued

```
Ser Pro Ser Pro Ile Arg Val His Thr Val Val Asp Arg Pro Gln Pro
    290                 295                 300

Met Thr His Arg Glu Thr Ala Pro Val Ser Gln Pro Glu Asn Lys Pro
305                 310                 315                 320

Glu Ser Lys Pro Gly Pro Val Gly Pro Glu Leu Pro Pro Gly His Ile
                325                 330                 335

Pro Ile Gln Val Ile Arg Lys Glu Val Asp Ser Lys Pro Val Ser Gln
            340                 345                 350

Lys Pro Pro Pro Pro Ser Glu Lys Val Glu Val Lys Val Pro Pro Ala
        355                 360                 365

Pro Val Pro Cys Pro Pro Pro Ser Pro Gly Pro Ser Ala Val Pro Ser
    370                 375                 380

Ser Pro Lys Ser Val Ala Thr Glu Glu Arg Ala Ala Pro Ser Thr Ala
385                 390                 395                 400

Pro Ala Glu Ala Thr Leu Pro Lys Pro Gly Glu Ala Glu Ala Pro Pro
                405                 410                 415

Lys His Pro Gly Val Leu Lys Val Glu Ala Ile Leu Glu Lys Val Gln
            420                 425                 430

Gly Leu Glu Gln Ala Val Asp Asn Phe Glu Gly Lys Lys Thr Asp Lys
        435                 440                 445

Lys Tyr Leu Met Ile Glu Glu Tyr Leu Thr Lys Glu Leu Leu Ala Leu
    450                 455                 460

Asp Ser Val Asp Pro Glu Gly Arg Ala Asp Val Arg Gln Ala Arg Arg
465                 470                 475                 480

Asp Gly Val Arg Lys Val Gln Thr Ile Leu Glu Lys Leu Glu Gln Lys
                485                 490                 495

Ala Ile Asp Val Pro Gly Gln Val Gln Val Tyr Glu Leu Gln Pro Ser
            500                 505                 510

Asn Leu Glu Ala Asp Gln Pro Leu Gln Ala Ile Met Glu Met Gly Ala
        515                 520                 525

Val Ala Ala Asp Lys Gly Lys Lys Asn Ala Gly Asn Ala Glu Asp Pro
    530                 535                 540

His Thr Glu Thr Gln Gln Pro Glu Ala Thr Ala Ala Ala Thr Ser Asn
545                 550                 555                 560

Pro Ser Ser Met Thr Asp Thr Pro Gly Asn Pro Ala Ala Pro
                565                 570


<210> SEQ ID NO 6
<211> LENGTH: 575
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Ser Ala Ala Thr His Ser Pro Met Met Gln Val Ala Ser Gly Asn
1               5                   10                  15

Gly Asp Arg Asp Pro Leu Pro Pro Gly Trp Glu Ile Lys Ile Asp Pro
            20                  25                  30

Gln Thr Gly Trp Pro Phe Phe Val Asp His Asn Ser Arg Thr Thr Thr
        35                  40                  45

Trp Asn Asp Pro Arg Val Pro Ser Glu Gly Pro Lys Glu Thr Pro Ser
    50                  55                  60

Ser Ala Asn Gly Pro Ser Arg Glu Gly Ser Arg Leu Pro Pro Ala Arg
65                  70                  75                  80
```

-continued

Glu Gly His Pro Val Tyr Pro Gln Leu Arg Pro Gly Tyr Ile Pro Ile
85 90 95

Pro Val Leu His Glu Gly Ala Glu Asn Arg Gln Val His Pro Phe His
100 105 110

Val Tyr Pro Gln Pro Gly Met Gln Arg Phe Arg Thr Glu Ala Ala Ala
115 120 125

Ala Ala Pro Gln Arg Ser Gln Ser Pro Leu Arg Gly Met Pro Glu Thr
130 135 140

Thr Gln Pro Asp Lys Gln Cys Gly Gln Val Ala Ala Ala Ala Ala Ala
145 150 155 160

Gln Pro Pro Ala Ser His Gly Pro Glu Arg Ser Gln Ser Pro Ala Ala
165 170 175

Ser Asp Cys Ser Ser Ser Ser Ser Ser Ala Ser Leu Pro Ser Ser Gly
180 185 190

Arg Ser Ser Leu Gly Ser His Gln Leu Pro Arg Gly Tyr Ile Ser Ile
195 200 205

Pro Val Ile His Glu Gln Asn Val Thr Arg Pro Ala Ala Gln Pro Ser
210 215 220

Phe His Gln Ala Gln Lys Thr His Tyr Pro Ala Gln Gln Gly Glu Tyr
225 230 235 240

Gln Thr His Gln Pro Val Tyr His Lys Ile Gln Gly Asp Asp Trp Glu
245 250 255

Pro Arg Pro Leu Arg Ala Ala Ser Pro Phe Arg Ser Ser Val Gln Gly
260 265 270

Ala Ser Ser Arg Glu Gly Ser Pro Ala Arg Ser Ser Thr Pro Leu His
275 280 285

Ser Pro Ser Pro Ile Arg Val His Thr Val Val Asp Arg Pro Gln Gln
290 295 300

Pro Met Thr His Arg Glu Thr Ala Pro Val Ser Gln Pro Glu Asn Lys
305 310 315 320

Pro Glu Ser Lys Pro Gly Pro Val Gly Pro Glu Leu Pro Pro Gly His
325 330 335

Ile Pro Ile Gln Val Ile Arg Lys Glu Val Asp Ser Lys Pro Val Ser
340 345 350

Gln Lys Pro Pro Pro Pro Ser Glu Lys Val Glu Val Lys Val Pro Pro
355 360 365

Ala Pro Val Pro Cys Pro Pro Pro Ser Pro Gly Pro Ser Ala Val Pro
370 375 380

Ser Ser Pro Lys Ser Val Ala Thr Glu Glu Arg Ala Ala Pro Ser Thr
385 390 395 400

Ala Pro Ala Glu Ala Thr Pro Pro Lys Pro Gly Glu Ala Glu Ala Pro
405 410 415

Pro Lys His Pro Gly Val Leu Lys Val Glu Ala Ile Leu Glu Lys Val
420 425 430

Gln Gly Leu Glu Gln Ala Val Asp Asn Phe Glu Gly Lys Lys Thr Asp
435 440 445

Lys Lys Tyr Leu Met Ile Glu Glu Tyr Leu Thr Lys Glu Leu Leu Ala
450 455 460

Leu Asp Ser Val Asp Pro Glu Gly Arg Ala Asp Val Arg Gln Ala Arg
465 470 475 480

-continued

Arg Asp Gly Val Arg Lys Val Gln Thr Ile Leu Glu Lys Leu Glu Gln
485 490 495

Lys Ala Ile Asp Val Pro Gly Gln Val Gln Val Tyr Glu Leu Gln Pro
500 505 510

Ser Asn Leu Glu Ala Asp Gln Pro Leu Gln Ala Ile Met Glu Met Gly
515 520 525

Ala Val Ala Ala Asp Lys Gly Lys Lys Asn Ala Gly Asn Ala Glu Asp
530 535 540

Pro His Thr Glu Thr Gln Gln Pro Glu Ala Thr Ala Ala Ala Thr Ser
545 550 555 560

Asn Pro Ser Ser Met Thr Asp Thr Pro Gly Asn Pro Ala Ala Pro
565 570 575

<210> SEQ ID NO 7
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide linker

<400> SEQUENCE: 7

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 8
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Lys Pro Ser Trp Arg Arg Tyr Arg Gly Trp Ser Arg Leu
1 5 10

<210> SEQ ID NO 9
<211> LENGTH: 85
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gtacaggggc tggag 85

<210> SEQ ID NO 10
<211> LENGTH: 64
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctg 64

<210> SEQ ID NO 11
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

aggggctgga 10

-continued

<210> SEQ ID NO 12
<211> LENGTH: 64
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtac 64

<210> SEQ ID NO 13
<211> LENGTH: 64
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtac 64

<210> SEQ ID NO 14
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctgg 65

<210> SEQ ID NO 15
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctgga 66

<210> SEQ ID NO 16
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtacaggggc t 71

<210> SEQ ID NO 17
<211> LENGTH: 72
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtacaggggc tg 72

<210> SEQ ID NO 18
<211> LENGTH: 68
<212> TYPE: DNA

-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggaga 68

<210> SEQ ID NO 19
<211> LENGTH: 73
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gta 73

<210> SEQ ID NO 20
<211> LENGTH: 73
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggacaag gta 73

<210> SEQ ID NO 21
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag 70

<210> SEQ ID NO 22
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtacaggggc tggag 75

<210> SEQ ID NO 23
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtacaggggc tggag 75

<210> SEQ ID NO 24
<211> LENGTH: 84
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

-continued

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gtacaggggc tgga 84

<210> SEQ ID NO 25
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtacaggggc tggag 75

<210> SEQ ID NO 26
<211> LENGTH: 82
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gtacaggggc tg 82

<210> SEQ ID NO 27
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtacaggggc tggag 75

<210> SEQ ID NO 28
<211> LENGTH: 85
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gtacaggggc tggag 85

<210> SEQ ID NO 29
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtacaggggc tggag 75

<210> SEQ ID NO 30
<211> LENGTH: 85
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gtacaggggc tggag 85

-continued

<210> SEQ ID NO 31
<211> LENGTH: 74
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

aaaaccagga gaagccgagg ctccccgaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gtac 74

<210> SEQ ID NO 32
<211> LENGTH: 85
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gtacaggggc tggag 85

<210> SEQ ID NO 33
<211> LENGTH: 85
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgctgaaag tggaagccat 60

cctggagaag gtacaggggc tggag 85

<210> SEQ ID NO 34
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

aaaaccagga gaagccgagg ctcccccaaa acatccagga gtgaagccat cctggagaag 60

gtacaggggc tggag 75

<210> SEQ ID NO 35
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

caggagaagc cgaggctccc ccaaaacatc caggagtgct gaaagtggaa gccatcctgg 60

agaaggtaca ggggctggca 80

<210> SEQ ID NO 36
<211> LENGTH: 64
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

aagccgaggc tcccccaaaa catccaggag tgaagccatc ctggagaagg tacaggggct 60

ggag 64

<210> SEQ ID NO 37
<211> LENGTH: 63
<212> TYPE: DNA

-continued

<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: n is a, c, g or t

<400> SEQUENCE: 37

agcngaggct cccccaaaac atccaggagt gaagccatcc tggacaaggt acaggggctg 60

gag 63

<210> SEQ ID NO 38
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

cgaggctccc ccaaaacatc caggagtgct gaaagtggaa gccatcctgg agaaggtaca 60

ggggctggag 70

<210> SEQ ID NO 39
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 39

aggctccccc aaaacatcca ggagtgaagc catcctggag aaggtacagg ggctggag 58

<210> SEQ ID NO 40
<211> LENGTH: 67
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40

ggctcccccа aaacatccag gagtgctgaa agtggaagcc atcctggaga aggtacaggg 60

gctggag 67

<210> SEQ ID NO 41
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 41

gctcccccaa aacatccagg agtgctgaaa gtggaagcca tcctggagaa ggtacagggg 60

ctggag 66

<210> SEQ ID NO 42
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 42

tcccccaaaa catccaggag tgaagccatc ctggagaagg tacaggggct ggag 54

<210> SEQ ID NO 43
<211> LENGTH: 62
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

-continued

ccccaaaaca tccaggagtg ctgaaagtgg aagccatcct ggagaaggta caggggctgg 60

ag 62

<210> SEQ ID NO 44
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

gagtgctgaa agtggaagcc atcctggaga aggtacaggg gctggag 47

<210> SEQ ID NO 45
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: n is a, c, g or t

<400> SEQUENCE: 45

atcnaggagt gaagccatcc tggagaaggt acaggggctg gag 43

<210> SEQ ID NO 46
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

gaaagtggaa gccatcctgg agaaggtaca ggggctggag 40

<210> SEQ ID NO 47
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

aggagtgaag ccatcctgga gaaggtacag gggctggag 39

<210> SEQ ID NO 48
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

agtggaagcc atcctggaga aggtacaggg gctggag 37

<210> SEQ ID NO 49
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

tgaagccatc ctggagaagg tacaggggct ggag 34

<210> SEQ ID NO 50
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

-continued

aaacatccag gagtgctgaa agtggaagcc a 31

<210> SEQ ID NO 51
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

aaacatccag gagtgmwgmm akyskrrrcc 30

1. A method of treating a patient suffering from, or, at risk of developing a muscle related disease or disorder comprising:

administering to the patient a therapeutically effective amount of an agent wherein the agent modulates expression or amount of BCL2-associated athanogene 3 (BAG3) molecules, proteins or peptides thereof in a target cell or tissue, as compared to a normal control.

2. The method of claim 1, wherein the agent comprises an expression vector expressing a BAG3 protein or active fragments thereof, oligonucleotides or combinations thereof.

3. The method of claim 2, wherein the expression vector further comprises a promoter, the promoter comprising an inducible promoter, a constitutive promoter, bicistronic promoter or tissue specific promoter.

4. The method of claim 1, wherein the expression vector comprises a viral vector, cardiotropic vector, plasmid, or a yeast vector.

5. The method of claim 4, wherein a cardiotropic vector comprises an adenovirus vector, an adeno-associated virus vector (AAV), a coxsackie virus vector, cytomegalovirus vector, Epstein-Barr virus vector, parvovirus vector, or hepatitis virus vectors.

6. The method of claim 1, wherein the expression vector is a cardiotropic pseudotyped viral vector.

7. (canceled)

8. The method of claim 1, wherein a muscle related disease or disorder is a cardiac disease or disorder.

9.-11. (canceled)

12. A method of diagnosing a patient with a cardiac disease or disorder comprising:

obtaining a biological sample from the patient,

measuring expression or quantifying BCL2-associated athanogene 3 (BAG3) polynucleotides, polypeptides or variants thereof, and/or identifying a mutation in a BAG3 molecule, wherein detection of decreased amounts or expression of BAG3 or a mutation in the BAG3 molecule, compared to normal healthy controls are diagnostic of a cardiac disease or disorder.

13. The method of claim 12, wherein a mutation comprises deletions or variants of BAG3 polynucleotides or polypeptides.

14. The method of claim 12, wherein a biological sample comprises: fluids, peptides, polypeptides, oligonucleotides, polynucleotides, cells, tissues or combinations thereof.

15. The method of claim 12, wherein an assay to measure expression or to quantify BCL2-associated athanogene 3 (BAG3) molecules and/or identifying a mutation in BAG3 molecule, comprises: immunoassays, phosphorylation assays, enzyme assays, bioassays, biochip assays, blots, hybridization assays, cell-based assays, high-throughput screening assays, chromatography, chemical assays, phage display assays, lab-on-a-chip, microfluidics based assays, microarrays, microchips, nanotube based assays, colorimetric assays, spectrophotometric assays or combinations thereof.

16.-24. (canceled)

25. A method of treating a subject at risk of or suffering from heart failure comprising: administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates expression or amount of a BCL2-associated athanogene 3 (BAG3) molecule, the agent comprising an expression vector encoding a BCL2-associated athanogene 3 (BAG3) molecule.

26. The method of claim 25, wherein the agent increases expression of BAG3 in a cell or tissue as compared to a baseline control.

27.-29. (canceled)

30. A method of treating a subject at risk of or suffering from idiopathic dilated cardiomyopathy comprising: administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of at least one agent which modulates expression or amount of a BCL2-associated athanogene 3 (BAG3) molecule.

31. The method of claim 30, wherein the agent increases expression of BAG3 in a cell or tissue as compared to a normal control.

32. The method of claim 30, wherein the agent comprises an expression vector encoding a BCL2-associated athanogene 3 (BAG3) molecule, oligonucleotides, polynucleotides, polypeptides, peptides, BAG3 protein BAG3 protein or active fragments thereof, or combinations thereof.

33. The method of claim 32, wherein the expression vector further comprises a promoter, the promoter comprising an inducible promoter, a constitutive promoter, bicistronic promoter or tissue specific promoter.

34. The method of claim 32, wherein the expression vector comprises a viral vector, cardiotropic vector, plasmid, or a yeast vector.

35. The method of claim 34, wherein a cardiotropic vector comprises an adenovirus vector, an adeno-associated virus vector (AAV), a coxsackie virus vector, cytomegalovirus vector, Epstein-Barr virus vector, parvovirus vector, or hepatitis virus vectors.

36. The method of claim 34, wherein the expression vector is a cardiotropic pseudotyped viral vector.

37.-49. (canceled)

* * * * *